PhD Thesis

STUDIES ON THE NEUROBEHAVIOURAL TOXIC EFFECTS OF THE MITOCHONDRIAL TOXIN, 3-NITROPROPIONIC ACID

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The Applicant's Publications referred to in the Thesis

I. **Lukács A.,** Szabó A., Nagymajtényi L. 2005. Behavioural and neurotoxicological effects following acute and subchronic administration of MK-801 in rats. Proceedings of 12th Symposium on Analitical and Environmental Problems pp. 201-205.

II. **Lukács A.,** Szabó A., Nagymajtényi L. 2005. Changes in neurobehavioural parameters and spontaneous cortical activity in rats after acute single and combined exposure by 3-nitropropionic acid and MK-801. Homeostasis 43: 169-172.

III. **Lukács A.,** Szabó A., Lengyel Zs. 2005. Central nervous effects of 3-nitropropionic acid and MK-801 elicited by acute single and combined administration in rats. Centr Europ J Occup Environ Med 11: 319-325.

IV. Szabó A., **Lukács A.,** Nagymajtényi L. 2005. Neurobehavioural and electrophysiological alterations in rats acutely treated with the mitochondrial toxin 3-nitropropionic acid and its functional antagonist, MK-801. Homeostasis 43: 173-178.

V. Szabó A., **Lukács A.,** Nagymajtényi L. 2005. Neurobehavioural and electrophysiological alterations in rats acutely treated with the neurodegenerative 3-nitropropionic acid and its functional antagonist, MK-801. Centr Europ J Occup Environ Med 11: 327-336.

VI. **Lukács A.,** Szabó A., Vezér T., Papp A. 2006. The acute effects of 3-nitropropionic acid on the behavior and spontaneous cortical electrical activity of rats. Acta Neurobiol Exp 66: 227-233. *Impact factor: 1.209*

VII. **Lukács A.,** Szabó A., Papp A., Nagymajtényi L. 2006. Behavioural alterations induced by acute and subacute administration of 3-nitropropionic acid in rats. Centr Europ J Occup Environ Med 12: 309-316.

VIII. **Lukács A.,** Szabó A. 2006. A 3-nitropropionsav akut és szubkrónikus adagolásával kiváltott magatartás-toxikológiai változások vizsgálata patkányban. Egészségtudomány 3-4: 202-208. (Az MHT Fiatal Higiénikusok II. Fórumán II. díjat nyert előadás alapján, felkérésre megírt közlemény.)

IX. Szabó A., Lengyel Zs., **Lukács A.,** Papp A. 2006. Studies on the neurotoxicity of arsenic in rats in different exposure timing schemes. Trace Elem Electroly 23: 193-198. *Impact factor: 0.571*

X. **Lukács A.,** Lengyel Zs., Institóris L., Szabó A. 2007. Subchronic heavy metal and alcohol treatment in rats: changes in the somatosensory evoked cortical activity. Acta Biol Hung 58 (3). Accepted for publication. *Impact factor: 0.636*

1. Introduction

1.1. Neurotoxic substances and neurodegenerative disorders

Exogenous substances exposing humans constitute a major cause of morbidity in general. Within all-cause morbidity, damage to the nervous system induced by environmental agents is an important field of epidemiological and toxicological research, due to the presence of neurotoxicants in air, drinking water, food etc.; originating partly from natural phenomena like mould infestation of foodstuffs (Johnson et al., 2000; Blumenthal, 2004) and arsenic in some artesian waters (Liu et al., 1994; Szabó et al., 2006), or from man-made emission of a great variety of chemicals. The resulting human nervous system diseases typically develop after long periods of exposure, are irreversible, and can be aggravated by adverse lifestyle factors (Maranelli et al., 1990; Lukács et al., 2007).

Also neurodegenerative disorders – such as Alzheimer's disease, Parkinson's disease (PD), Huntington's disease (HD), amyotrophic lateral sclerosis and cerebellar degeneration – typically occur in elderly patients (Vécsei et al., 1998). The term, neurodegenerative disorders, implies that our knowledge of the cause or pathogenesis of these diseases is incomplete. In some, like PD, environmental influences such as chronic low-level intoxication by manganese (Barceloux, 1999) or by some pesticides (Kamel and Hoppin, 2004) have been proposed as possible causative factors. In others, like HD, genetic origin has been evidenced (see 1.2) – but in the animal modelling of this disease, which is crucial in learning more about the pathomechanism and the possible ways of therapy, and is the topic of this Thesis, an environmental factor, viz. food-borne poisoning by a fungal neurotoxin, has been playing an important role.

The present demographic trend of the population, in Hungary and elsewhere, causes a significant increase in the incidence of the neurodegenerative disorders (Aronson, 1996). Hence, these diseases have been attracting great research interest, because drugs, are able to influence their pathogenesis, are not available, and because their pathomechanism can be studied, as mentioned above, in animal models. All neurodegenerative disorders are characterized by histological damage and neuronal cell

death, caused by metabolic compromise, oxidative stress and excitotoxicity (Alexi et al., 2000).

In the pathogenesis of HD, excitotoxicity may play an important role. Excitotoxicity includes a dysfunction of excitatory amino acid (EAA) neurotransmission, usually a hyperstimulation of glutamate receptors. Glutamate receptors mediate most of the fast excitatory synaptic transmission in the mammalian central nervous system (CNS). Based on their pharmacological properties, they have been classified into three major receptor channel subtypes, the α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) subtype, the kainate subtype, and the N-methyl-D-aspartate (NMDA) subtype (Yamakura and Shimoji, 1999). The striatum is the most glutamaceptive region in the central nervous system (receiving ample glutamatergic cortical innervation as the first stage of the cortex-striatum-thalamus-cortex loop) and is thus predisposed to excitotoxicity. NMDA receptors are abundant in the hippocampus, which is involved in locomotion, learning and memory (Bast et al., 2005); and in the striatum, which regulates sensorimotor gating (Koch and Schnitzler, 1997). Excess activation of NMDA receptors induces apoptosis or necrosis of the cells expressing them, which will result in behavioural and functional disorders. Two mechanism of excitotoxicity may be relevant: excess activation of EEA receptors, or abnormal function of ion pumps caused by impairment of energy metabolism. Quinolinic acid and other agonists of NMDA receptor induce a pattern of striatal degeneration most similar to that found in HD, and provide a model of the disease.

Mitochondrial toxins also lead to a pattern of striatal atrophy similar to that of HD. 3-nitropropionic acid (3-NP) and malonic acid, e.g., interfere with ATP synthesis and cause a deficit of energy leading to striatal lesions. The striatum is also enriched in dopamine (DA) due to innervating nigrostriatal and nigropallidal dopaminergic afferents (Alexi et al., 2000). DA itself can be neurotoxic, and the mechanism of 3-NP toxicity involves DA.

Beyond uncleared pathogenesis, the therapy of neurodegenerative disorders is also problematic. A large number of therapeutic approaches have been explored, and numerous neuroprotective agents have been tested in animal models. Beyond monitoring of therapic drug effects, animal models may contribute to early disease recognition, because modelling the behavioural disturbances, developing in the initial state of

neurodegenerative disorders in humans, can contribute to earlier and more specific diagnosis. Hence, based on our previous electrophysiological investigations (Szabó and Papp, 2003), behavioural model of HD was set up based on systemic administration of 3-NP. Locomotor activity, motor coordination and acoustic startle response (ASR) were chosen to be tested, because DA and glutamate, both of which play an essential role in the pathomechanism of neurodegenerative disorders, are also involved in these behavioural phenomena. Damage of the basal ganglia – which regulate motor and affective behaviour by dopaminergic and glutamatergic projections (Alexi et al., 2000) – is detectable by open field (OF) tests. The impairment of nigrostriatal dopaminergic system also influences rotarod performance (Rozas and García, 1997). In the ventral striatum, glutamatergic and dopaminergic neurotransmitter systems play a role in the regulation of pre-pulse inhibition (PPI) of ASR (Koch, 1999) therefore, loss of striatal neurons, observed in neurodegenerative diseases, may be evaluated by ASR test.

1.2. Huntington's disease

1.2.1. History, background and description of Huntington's disease

Huntington's disease (HD) is an autosomal, dominant hereditary neurodegenerative disorder which is characterized by progressive dementia and involuntary abnormal choreiform movements (Borlongan et al., 1997b). The illness was delineated as a separate disease by George Huntington in 1872. Huntington described key features of HD, separating it from other forms of chorea, which is a state of excessive, involuntary movements, irregularly timed, randomly distributed, and abrupt (Barbeau et al., 1981). The word 'chorea' is derived from Latin choreus (Greek: choros) and it refers to dancing. George Huntington identified that HD manifests not only in chorea, but defined the disease as an adult onset, hereditary, progressing, fatal disorder which causes also psychological features. So the earlier term „Huntington's Chorea" was changed „Huntington Disease" (Alexi et al., 2000). This pathological state was known as St. Vitus' dance in the late 17th century in Europe, because of the movements due to hyperkinesis are similar to dancing. In Salem, Massachusetts, in 1692, many of the „witches" accused of being possessed by the devil are now believed to have had HD. Numerous hypothesis have been devised to explain the afflicted behaviour, which was characterized by disorderly speech, odd postures, gestures and convulsive fits. Some speculate that the

„witches" had HD (Maltsberger, 1961), but others suggest that ergot poisoning play a role in their physical states (Woolf, 2000). The genetic transmission of HD became evident in that all patients with this disease were descendants of two ancestors, born in England and emigrated to Salem in 1630 (Kandel et al., 1996).

The HD gene and its mutation was identified by The Huntington's Disease Collaborative Research Group in Boston, and was published in the *Cell* in 1993 (The Huntington's Disease Collaborative Research Group, 1993). The *huntingtin* gene is on the telomere of the short arm of chromosome 4, also known as IT 15 (interesting transcript 15), which consists of 67 exons.

The mutation that causes HD is an expansion of a part of the HD gene. The expanded part consists of tandemly repeated CAGs, which is the genetic code for amino acid glutamine. In normal individuals, CAG repeat is between 9 and 35 (mean=19), which does not cause HD gene mutation. Individuals with repeats ranging from 36-39 may or may not develop HD. With 40 or more repeats, the disease will surely develop. The juvenile-onset form of HD – which is associated with a greater severity of symptoms – shows an expansion of 50-70 CAG repeats or more (Snell et al., 1993).

The „healthy" *huntingtin* plays a role in the transport mechanisms within the cells and prevents the apoptosis of neurons in the central nervous system. In contrast, the abnormal *huntingtin* inhibits the function of the normal gene product and causes apoptosis, aggregating the brain-derived neurotrophic factor (BDNF) which is indispensable to the life of the neurons. Moreover, it stimulates procaspase-9 enzyme, which participates in the apoptosis. The mechanism of selective neurodegeneration of the striatum that occurs due to this mutation is not understood (Alexi et al., 2000). The multitude of factors probably contributing to this selective vulnerability include differing levels of expression of huntingtin, differences in the density of NMDA receptors, and the degree of cortical innervation (Cepeda et al., 2007). In HD, DA release is also affected because of a dramatic loss of striatal GABAergic neurons. GABA, an inhibiting neurotransmitter, modulates DA release in both striatal projection and interneurons.

The prevalence rate of clinically identified HD is 1/10,000 in most West-European countries and in North-American and Hungarian population. But markedly less people are concerned with HD in Asia, Africa and Finland (Jakab et al., 1999). This may be explained so that the Hungarian population is a melange of different peoples, but the

Finnish were genetically more isolated in the last centuries, so the frequency of HD is lower (Jakab et al., 1999).

Clinical symptoms of HD begin at 40-45 years of age with behavioural disturbances, developing very rapidly and leading to death on average after 17 years of disease. Clinical signs of the disease show a three-part picture with motor abnormalities, psychiatric disturbances and profound cognitive impairment (Brouillet et al., 1999).

The disease generally begins with motor symptoms, progressing over a 10-15 year period from a hyperkinetic to an akineto-rigid syndrome. The earliest motor signs are eye movement abnormalities followed by orofacial dyskinesis. Dyskinesis extends to head, neck, trunk and limbs, and finally generalized chorea appears. Eventually the initial hyperkinetic syndrome is replaced by a more hypokinetic syndrome in which bradykinesia and rigidity predominate.

A progressive lesion of the striatum disconnects the prefrontal cortex from the basal ganglia circuitry, interrupting the flow of information arising from the cerebral cortex. In the initial state of the disease, frontal-type dementia is observed; in addition, primary or secondary prefrontal cortical lesions may also be responsible for the cognitive deficits (Diamond et al., 1992). Experimental data in non-human primates show that selective lesions of the caudatum – similar to what is observed in the prefrontal cortex – induce behavioural alterations. Psychiatric alterations, like aggressivity and depression, are also marked in patients, later psychosis and hallucination aggravate the features of HD.

1.2.2. Animal model of Huntington's disease

Most of the HD models used today are based on the action of mitochondrial toxins. Administration of mitochondrial toxins causes excitotoxic lesions similar to that of HD. Damage is found especially in the striatum, with concomitant damage in the hippocampus, thalamus, and cortex (Kodsi and Swerdlow, 1997). Because of the neuropathological and behavioural similarities between the effects of systemic administration of 3-NP in experimental animals and HD, 3-NP has been proposed as a HD model substance.

Administered systemically, 3-NP induces selective bilateral striatal degeneration in rodents and primates including humans (Beal et al., 1993). Beyond the factors outlined above, there are some specific mechanisms underlying the selective vulnerability of striatum: the sensitivity of the lateral striatal artery (Kawamura et al., 1991), high levels of glutamate transporter activity in the striatum (Mcbean, 1994), and dopaminergic toxicity due to the concentrated location of D_2 receptors in the lateral striatum (Ben-Shachar et al., 1995). Beyond histological effects, bilateral striatal degeneration was shown to cause neurobehavioural alterations (Borlongan et al., 1995). These included alteration of locomotor activity (Borlongan et al., 1997b) and of the sensorimotor gating mechanism (Seaman, 2000). Manipulating the experimental scheme of systemic 3-NP administration can differentially produce locomotor hyperactivity as seen in early HD, or hypoactivity as seen in advanced and early-onset HD (Alexi et al., 2000). Dopaminergic neurons in the substantia nigra play a role in the control of motor and affective behaviour by basal ganglia (Alexi et al., 2000). Systemic administration of 3-NP also causes startle reflex abnormalities and reduced sensorimotor gating (Kodsi and Swerdlow, 1997). In patients with HD, impaired PPI can be observed (Swerdlow et al., 1995). Recent experiments revealed a complex role for glutamate and DA receptors in the nucleus accumbens (NAC) in the regulation of PPI.

1.3. 3-nitropropionic acid

1.3.1. Poisoning by 3-NP

The agent 3-NP (Fig. 1; also known β-nitropropionic acid, bovinocidin, hiptagenic acid) is a light yellow; fine, crystalline solid. 3-NP has a short plasma half life, in rats the plasma concentration of 3-NP fell to 10 % of peak value within 40 minutes (Ray, 1999). The acute intraperitoneal LD_{50} for rats is in the range of 60-80 mg/kg (Burdock et al., 2001). 3-NP is absorbed in the gastrointestinal tract, enters the circulation, and is metabolized to nitrite, although some may bind the enzyme succinate dehydrogenase (SDH) upon oxidation (Burdock et al., 2001). 3-NP is metabolized in the liver, data of excretion of 3-NP are not available.

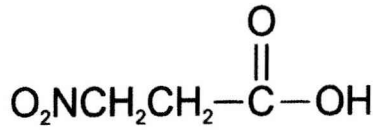

Figure 1 Chemical structure of 3-nitropropionic acid.

In the Western states of North America, the grazing animals are often poisoned by 3-NP (Williams and James, 1975; Niknam et al., 2003). 3-NP is a neurotoxic product of several species of *Astragalus* (Leguminosae), growing on grasslands. Worldwide, more than 450 species and varieties of *Astragalus* are known to contain either 3-nitropropanol or 3-NP (but not both), the former being converted to 3-NP by alcohol dehydrogenase. In these plants, 3-NP was detected from <1 ppm to 26000 ppm as 3-NP, 3-nitropropanol or as NO_2, depending on the method of analysis (Burdock et al., 2001). 3-NP is produced also in the *Corynocarpus laevigatus,* a tree in New Zealand; and there are reports of toxicity in cattle and pigs; and in honeybees, attributed to its flowers, with 20 % mortality (Bell, 1974). Grazing of 3-NP containing plants causes poisoning in animals only with one stomach, and not in ruminants, where 3-NP is decomposed in the rumen. The symptoms of acute poisoning in animals are weakness, incoordination, cyanosis and emphysema. Microscopic lesions are observed in the lung and central nervous system. Chronic poisoning is characterized by weight loss and irreversible hind limb ataxia (James et al., 1980).

One of two sources of human 3-NP poisoning is karaka tree nuts, because 3-NP is produced also in the fruit of *Corynocarpus laevigatus* (Majak and Benn, 1994). There is a report describing toxicity in children following consumption of the unprocessed nuts (Burdock et al., 2001). Eating food contaminated with fungi offers more exposure of 3-NP, also responsible for a number of cases of human poisoning. Numerous species of *Arthrinium, Aspergillus* and *Penicillium* fungi can produce the toxin, growing on sugarcane (higher hazard because of higher sugar content), soybeans, peanuts and curds if they are stored under damp conditions (Johnson et al., 2000). Accidental poisonings were first published by Liu et al. (1989), who reported exposure by mouldy sugar cane. In China, in the period of 1972-1989 more than 800 human cases were reported with 88 deaths. 2-3 hours after the consumption of infested food, gastrointestinal symptoms appeared which usually progressed only to headache. In severe cases, convulsions,

rigidity and coma developed by 3-18 hours. Late onset dystonia was seen in several cases 7-40 days after the poisoning (Burdock et al., 2001). In humans undergoing high dose acute and chronic intoxication, permanent brain damage was observed. Especially the caudatum and claustrum are affected, besides bilateral hypodensity is observed in the putamen and globus pallidus (Spencer et al., 1993). These observations conduced to development of the model of the disease, because these areas are also affected in case of human HD.

1.3.2. The toxicity of 3-NP in experimental studies

3-NP irreversibily and selectively inhibits the enzyme SDH due to its structural similarity of succinic acid. This enzyme is a component of complex II of mitochondrial electron transport chain in the Szent-Györgyi-Krebs cycle. In normal condition, complexes from I to V (ATP synthase) form the dominant pathway of ATP synthesis. Complex II plays an important role by dehydrogenating succinic to fumaric acid (Lee and Chang, 2004). The chemical structure of 3-NP is isoelectronic with that of succinate and can bind to the catalytic site of SDH. Inhibition of SDH, therefore, causes energy impairment in the central nervous system (Alexi et al., 1998), but also in other parts of the body (Alexi et al., 2000). SDH inhibition is dependent on the chemical breakdown of 3-NP and the rate of resynthesis of new, uninhibited enzyme (Ray, 1999). Reduced ATP production leads to decreased pumping activity of Na^+/K^+ ATPase, so that intracellular K^+ level will decrease, and Na^+ increase, according to their electrochemical gradient. Increased intracellular Na^+ concentration then reverses the function of the Na^+/Ca^{2+} exchanger, leading to Ca^{2+} influx into the neurons.

Another mechanism of increased intracellular Ca^{2+} concentration is the activation of NMDA receptors. Depolarization due to inhibited Na^+/K^+ ATPase eliminates the Mg^{2+} block of NMDA channels, activating them this way, and the opening of NMDA channels results in increase of intracellular Ca^{2+} concentration (Lee et al., 2002). Beyond the extracellular Ca^{2+} influx via NMDA and non-NMDA (such as AMPA) bound and voltage-gated calcium channels (VGCCs), release of Ca^{2+} from the endoplasmatic reticulum (which is one of the major Ca^{2+} buffering systems in neurons) also contributes to the dramatic elevation of intracellular Ca^{2+} concentration. Elevated intracellular Ca^{2+}

concentration activates Ca^{2+}-dependent enzymes, such as proteases, lipases, kinases, leading to apoptosis (Portera-Caillau et al., 1995) or necrosis (Coyle and Puttfarcken, 1993) depending on the extent of cellular ATP depletion. An increase in intracellular Ca^{2+} concentration also leads to the activation of nitric oxide synthase (NOS) and to reactive oxygen species (ROS) production. The nitric oxide (NO) produced by NOS reacts with superoxide anions to form peroxynitrite, which is toxic to neurons and can further deplete the neuronal ATP pool. Increased ROS impairs mitochondrial function and calcium buffering activity, contributing thus to cell necrosis or apoptosis (Lee and Chang, 2004) (Fig. 2).

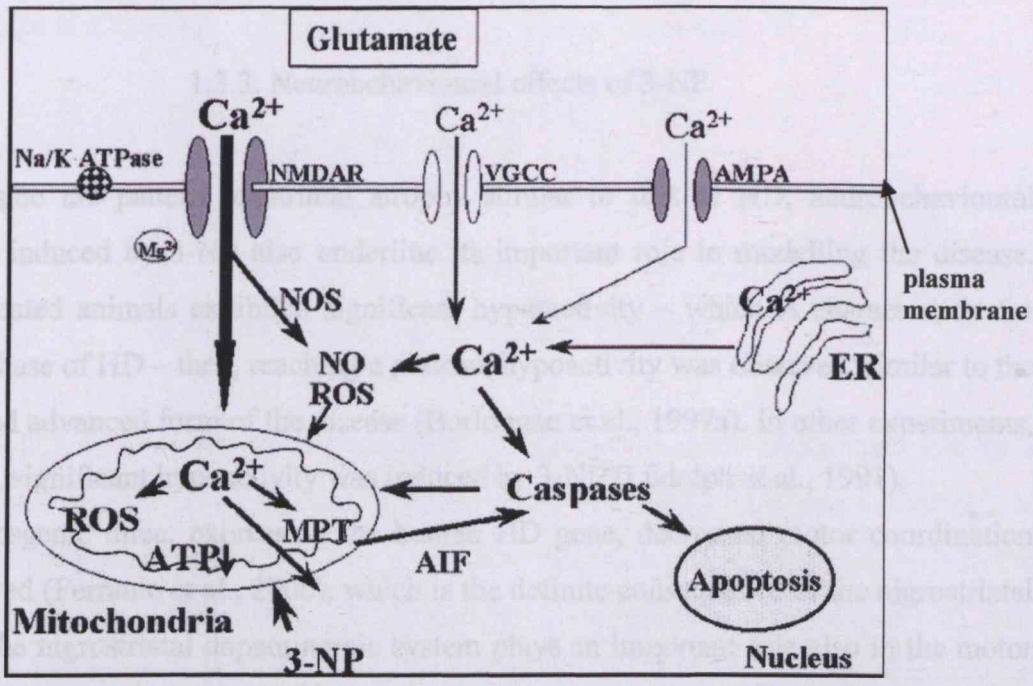

Figure 2 The mechanism of 3-NP neurotoxicity (Lee and Chang, 2004).

Abbreviations: AIF: apoptosis inducing factor, ER: endoplasmic reticulum, NMDAR: N-methyl-D-aspartate receptor, NO: nitric oxide, NOS: nitric oxide synthase, MPT: mitochondrial permeability transition, ROS: reactive oxygen species, VGCC: voltage-gated calcium channel

The striatum receives massive glutamatergic and dopaminergic innervations. Dopaminergic input comes from the pars compacta of the substantia nigra. The nigrostriatal presynaptic D_2 receptors have autoreceptor function, so they may inhibit the further DA release. In the rat, motor areas of the cortex makes direct synaptic connections with striatal neurons (Hersch et al., 1995). The D_2 receptors on these corticostriatal terminals may be involved in presynaptic inhibition of glutamate release. The explanation of the toxicity mechanism of 3-NP, proposed by Nishino et al. (1997) is based on the

dopaminergic, rather than glutamatergic, functions. D_2 receptors, found in about 45% of striatal neurons, are especially abundant in the laterocentral part of the striatum (Hersch et al., 1995). Prior to cell death, dopaminergic neurons can be over-excited by the increased Ca^{2+} influx and can release DA (Castro et al., 1996). Johnson et al. (2000) measured an increase of DA in the striatum, and suggested that the inhibition of SDH lead to decreased energy production, and this cerebral energy deficit resulted in an elevation of DA concentration. The interaction between DA and glutamate depends on the area in question, but it is believed that glutamate regulates DA release in the striatum (David et al., 2005).

1.3.3. Neurobehavioural effects of 3-NP

Beside the pattern of striatal atrophy similar to that of HD, neurobehavioural alterations induced by 3-NP also underline its important role in modelling the disease. Acutely treated animals exhibited significant hyperactivity – which is characteristic for the early phase of HD – then, reaching a plateau, hypoactivity was observed, similar to the juvenile and advanced form of the disease (Borlongan et al., 1997a). In other experiments, permanent, significant hypoactivity was induced by 3-NP (Ludolph et al., 1991).
In HD transgenic mice, expressing the human HD gene, decreased motor coordination was observed (Ferrante et al., 2000), which is the definite consequence of the nigrostriatal damage. The nigrostriatal dopaminergic system plays an important role also in the motor performance of rats. Seaman (2000) described reduced sensorimotor gating after systemic administration of 3-NP in rats, which was also observed in human HD (Swerdlow et al., 1995). Psychomotor inhibition is influenced by dopaminergic transmission, but other neurotransmitter systems (glutamatergic, cholinergic), too, play a role in the regulation of gating mechanism.

In the background of 3-NP induced neurobehavioural alterations, both glutamatergic and dopaminergic functions are supposed. In order to investigate the action of 3-NP on these neurotransmitter systems, drugs were chosen, which are known to act on these. Several drugs showed neuroprotective effect in 3-NP treated rats, including antagonists of NMDA receptors, such as MK-801 (Schulz et al., 1996b; Lee et al., 2000). One aim of this study was to test the effects of 3-NP on glutamatergic system via

combination with MK-801, a direct inhibitor of the NMDA glutamate receptor. The involvement of DA in the consequences of 3-NP exposure (Koutouzis et al., 1994; Alexi et al., 2000) gave the reason of applying a D_2 receptor agonist (quinpirole) and an antagonist (sulpiride) in the experiments with 3-NP.

1.4. MK-801

The drug MK-801 (Fig. 3; dizocilpine maleate) is a white, crystalline solid. The half-life time of the drug is around 2 hours (Horváth et al., 1997). The acute intravenous LD_{50} for mice is 30 mg/kg.

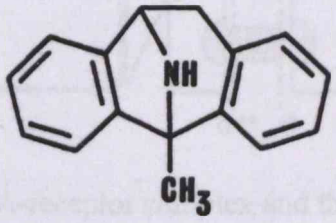

Figure 3 Chemical structure of MK-801.

MK-801 is a selective, non-competitive NMDA receptor antagonist, and is a potent, common anticonvulsant that exhibits both anxiolytic and sympathomimetic properties (Wong et al., 1986). MK-801 is an open-channel blocker, which can bind to the inside of the NMDA channel (Fig. 4), and can block the influx of extracellular Ca^{2+} into the cell. On the basis of this effect on NMDA receptors, MK-801 was expected to be neuroprotective in excitotoxic impairment induced e.g. by 3-NP. MK-801 was thus tested by administering it before and after 3-NP (see Table I). However, this agent has psychotomimetic actions in humans, and itself causes morphological damage to neurons in the cerebral cortex of rats. Olney et al. (1990) reported that MK-801 induced acute pathomorphological changes in neurons of the retrosplenial cortex when administered subcutaneously to adult rats in relatively low doses. Hence, its own neurotoxic effect was also investigated, following intraperitoneal injection.

There is some evidence that MK-801 has an effect on the dopaminergic system, but its effect on DA release is rather equivocal. Some reported that MK-801 increased DA metabolism in the striatum (Löscher and Hönack, 1992; Hiramatsu et al., 1989), while others found little effect of MK-801 on striatal metabolism (Araki et al., 2001). Takahashi

et al. (1996) reported that MK-801 blocks DA liberation in the striatum. According to several studies (Segovia et al., 1997; Morari et al., 1993) NMDA agonists produced a release of DA in the striatum; MK-801 therefore should block this directly . The possible dopaminergic effect of MK-801 was tested via combined administration with a D_2 receptor agonist and an antagonist (see below).

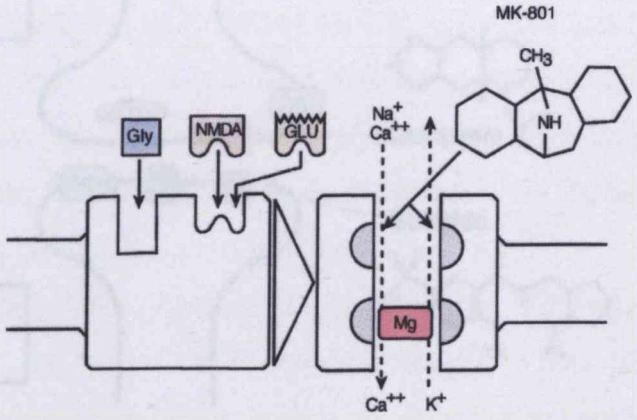

Figure 4 The NMDA-receptor complex and the action of MK-801.
Abbreviations: Gly: glycine, GLU: glutamate

1.5. Quinpirole and sulpiride

The agent quinpirole (QP) is a white solid, reported to be a selective agonist at D_2 receptor sites. D_2 receptors on corticostriatal dopaminergic terminals play a key role in autoregulation of DA activity and DA release (Hersch et al., 1995). Decreased DA release is expected by the stimulation of these inhibitory autoreceptors (Fig. 5) with QP.

Sulpiride (SP; synonyms: abilit, aiglonyl, dobren, misulvan, omperan) is a white solid, the acute subcutaneous LD_{50} for rats is 360 mg/kg. SP is a benzamide derivative with antipsychotic properties, used for treating different neuropsychiatric disorders. SP is an atypical neuroleptic (acting more or less selectively in the mesolimbic dopaminergic system), and decreases the levels of DA in the NAC soon after its administration (Fujiwara, 1991). SP acts selectively as a dopamine D_2 receptor antagonist in the brain (Fig. 5), its effects on other neuronal systems being extremely limited (Jenner and Marsden, 1982).

Since the dopaminergic system is also affected in neurodegenerative disorders, we have also tested whether pretreatment with the D_2 agonist QP and D_2 antagonist SP can influence or prevent the alterations induced by 3-NP.

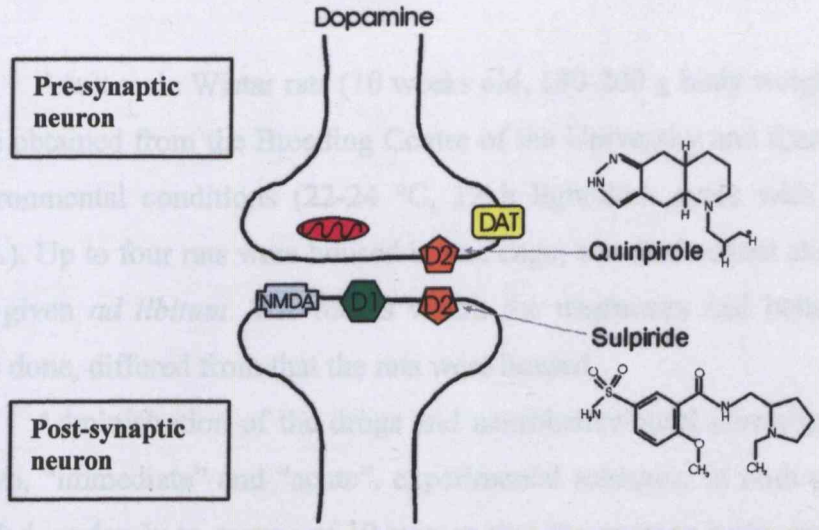

Figure 5 The action of quinpirole and sulpiride on D_2 receptors (Riddle et al., 2006).

Abbreviations: DAT: dopamine transporter; NMDA: N-methyl-D-aspartate receptor.

1.6. Aims of the study

At present, the existing HD models are based on the same histological damage, in the same area of the central nervous system. 3-NP-caused neurobehavioural alterations, although described in the literature, were not systematically connected to the toxicity mechanism of 3-NP and to its effects on neurotransmitter systems. The aim of the study was to reveal the mechanism of neurobehavioural alterations more in detail, thereby contributing to the improvement of the HD model, in order to investigate the potential protective agents on behavioural, and not only histological, endpoints.

The role of dopaminergic and glutamatergic transmission in behavioural phenomena, and the effect of 3-NP on the former; supported such an experimental approach. Spontaneous locomotor activity, motor coordination and ASR/PPI – phenomena known to be influenced by 3-NP – were investigated in short-time exposure experiments, due to the short plasma half life of the toxin.

2. Methods

2.1. Animals, drugs and general layout of the experiments

Adult male Wistar rats (10 weeks old, 180-200 g body weight) were used. The rats were obtained from the Breeding Centre of the University and then kept under controlled environmental conditions (22-24 °C, 12 h light/dark cycle with light starting at 6.00 A.M.). Up to four rats were housed in one cage; standard rodent chow and drinking water was given *ad libitum*. The rooms where the treatments and behavioural investigations were done, differed from that the rats were housed.

Administration of the drugs and neurobehavioural investigations were performed in two, "immediate" and "acute", experimental schemes. In both schemes, the rats were divided randomly to groups of 10 rats, so that the average body weight in the groups was similar. The time scheme of the experiment was as follows. Baseline behavioural data (OF, rotarod, ASR and PPI) were recorded preceding the treatment. In the immediate scheme, the rats were treated with the drugs, one by one, with 20 min interval between each two rats, and 30 min after treatment the behavioural tests were repeated. In the acute scheme, the behavioural investigations were done 24 h after drug administration.

Drugs were purchased from Sigma Aldrich GmbH (Steinheim, Germany) and were dissolved in saline, except sulpiride which was dissolved in dimethyl sulfoxide (DMSO), to 1.0 ml/kg body weight administration volume. 3-NP (20 mg/kg) and MK-801 (0.8 mg/kg) was given the rats by intraperitoneal (ip.) injection, alone or combined. In the combination groups, 30 minutes were left between injecting the first and the second drug. Quinpirole (5 mg/kg) or sulpiride (80 mg/kg) was injected subcutaneously (sc.) 15 min before the administration of 3-NP or MK-801. Controls were injected with saline. Drug doses and time of injections were based on Szabó and Papp (2003) and Lukács et al. (2006), and on literature data (Kodsi and Swerdlow, 1997; Nishino et al., 1997). Groups, treatments, doses and time course of the experiments are given in Table I.

Table I Groups, treatments, doses, and time course of immediate (A) and acute (B) experimental schemes.

A/

Groups	Treatments		Tests (time after first administration)		
	0'		30'	43'	50'
Control	Saline		*OF*	*Rotarod*	*ASR, PPI*
3-NP	3-NP: 20 mg/kg ip.				
MK-801	MK-801: 0.8 mg/kg ip.				
	0'	30'	60'	73'	80'
3-NP+ MK-801	3-NP: 20 mg/kg ip.	MK-801: 0.8 mg/kg ip.	*OF*	*Rotarod*	*ASR, PPI*
MK-801+ 3-NP	MK-801: 0.8 mg/kg ip.	3-NP: 20 mg/kg ip.			
	0'	15'	45'	58'	65'
QP+ 3-NP	QP: 5 mg/kg	3-NP: 20 mg/kg	*OF*	*Rotarod*	*ASR, PPI*
QP+ MK-801	QP: 5 mg/kg	MK-801: 0.8 mg/kg			
SP+ 3-NP	SP: 80 mg/kg	3-NP: 20 mg/kg			
SP+ MK-801	SP: 80 mg/kg	MK-801: 0.8 mg/kg			

B/

Groups	Treatments		Tests (time after first administration)		
	0 h 0'		24 h 0'	24 h 13'	24 h 20'
Control	Saline		*OF*	*Rotarod*	*ASR, PPI*
3-NP	3-NP: 20 mg/kg ip.				
MK-801	MK-801: 0.8 mg/kg ip.				
	0 h 0'	0 h 30'	24 h 30'	24 h 43'	24 h 50'
3-NP+ MK-801	3-NP: 20 mg/kg ip.	MK-801: 0.8 mg/kg ip.	*OF*	*Rotarod*	*ASR, PPI*
MK-801+ 3-NP	MK-801: 0.8 mg/kg ip.	3-NP: 20 mg/kg ip.			
	0 h 0'	0 h 15'	24 h 15'	24 h 28'	24 h 35'
QP+ 3-NP	QP: 5 mg/kg	3-NP: 20 mg/kg	*OF*	*Rotarod*	*ASR, PPI*
QP+ MK-801	QP: 5 mg/kg	MK-801: 0.8 mg/kg			
SP+ 3-NP	SP: 80 mg/kg	3-NP: 20 mg/kg			
SP+ MK-801	SP: 80 mg/kg	MK-801: 0.8 mg/kg			

Abbreviations: 3-NP: 3-nitropropionic acid, QP: quinpirole, SP: sulpiride, OF: open field, ASR: acoustic stratle response, PPI: pre-pulse inhibition.

2.2. Behavioural tests

2.2.1. Spontaneous locomotor activity in open field

To record the animals' movement has a considerable importance and is very useful in numerous behavioural and neurophysiological studies (Kao et al., 1995). Mice and rats are attracted by novel stimuli and spend long periods exploring when exposed to a novel environment (Crusio, 2001). According to Crusio's definition (2001), "exploration is evoked by novel stimuli and consists of behavioural acts and postures that permit the collection of information about new objects and unfamiliar parts of the environment". Consequently, it is not appropriate to equate exploratory behaviour with "activity", because almost all behaviours are "activities", but not all of them can be classified as exploratory. The rats' spontaneous exploratory activity was investigated in an OF apparatus (Conducta 1.0 System, Experimetria Ltd.) in 10-minute sessions. The animals were first allowed to accommodate in the testing room for 20–30 min, and then the rats were placed in the OF box (48×48×40 cm) equipped with several arrays of infrared sensors (at floor level and in 12 cm height). Motility parameters – ambulation distance, time and count; rearing time and count; local time and count; immobility time and count – were computed on the basis of infrared beam interruptions. In the evaluation of horizontal movement, the rat was modelled with a rectangular object, and the shift of its centre was recorded. If there was more than 40 mm shift in the location of interrupted beams at the floor level during a time unit of 1 s, ambulatory movement (running) was recorded, if there was less shift, local activity (grooming), and if there was no shift at all, immobility. Vertical activity (rearing) was recorded if beams at the floor level and at the higher level were interrupted simultaneously. These four forms of activity were mutually exclusive so that the time spent by them covered totally the 600 seconds of the session (referred to as "total open field activity" in Tables II, and VI). The OF investigation was performed from 8.00 A.M. to 2.00 P.M. in both experimental schemes, followed by the rotarod test and ASR/PPI.

2.2.2. Motor coordination in rotarod

The rotarod method has been originated by a paper of Dunham and Miya (1957). It has become the most widely used technique for investigating the effects of drugs which are potentially active on motor coordination. This test is based on the fact that, in animals, application of drugs acting on the dopaminergic system causes decreased motor skills (Rozas et al., 1998; Sedelis et al., 2001). Before the beginning the experiment, the rats received a preliminary training on the rotarod (ROTA-ROD for rats 47700, Ugo Basile, Italy) with the rotational speed accelerating day after day, in the range 1-10 rounds per minute (r.p.m.), on 5 consecutive days. The head of the rat was placed against the direction of the rotation, so that the animal had to move forward in order to stay on the top of the rod (Rozas and Garcia, 1997). At the end of the training, they were expected to stay on the rod for about 200 sec. For rotarod test, the time was measured that the rats were able to spend on the rod, accelerating evenly from 1 to 10 r.p.m, during the 300 sec session. When a rat fell off onto the plate below, the plate tripped and a magnetic switch was activated, thereby recording the animal's endurance time in seconds.

2.2.3. Acoustic startle response and pre-pulse inhibition in ASR

ASR is a fast involuntary contraction of facial and body muscles evoked by sudden, unexpected and intense acoustic stimuli (Koch and Schnitzler, 1997). Changes of the ASR by systemic application of drugs are widely used to assess the drugs' effects on sensorimotor reactivity in animals and humans (Koch, 1999). The test was performed within a sound-proof plexiglas chamber (16×28×18 cm; Responder X System, Columbus Instrument, Ohio, USA), where the rats were put into, one by one, on the top of a load cell platform. A continuous white background noise (65 dB) was delivered from the loudspeaker mounted 10 cm from the test cage. Following a 10-15 min accomodation period, the rats were exposed to a series of 10 startling stimuli (5 kHz, 110 dB, 200 ms, 15 s interval). The vertical force associated with the startle response was measured by the load cell (a piezoelectric force transducer).

Following a 15 minutes recovery time, another session of 10 stimuli was started. This time a low-amplitude stimulus, a pre-pulse (1 kHz, 73 dB, 500 ms) was presented just before the startling stimulus (inter-stimulus time: 200 ms). This "warning" pulse

attenuates the level of the startle response, and this normal suppression of the ASR by a preceding stimulus is termed pre-pulse inhibition (PPI) (Braff and Geyer, 1990).

In both tests, the rat's muscle twitch exerting more than 50 g force on the platform was accepted as "noise-positive" response. Beside counting these events, three parameters were calculated from the recorded force-time course of the ASR: *peak amplitude* of the startle response, its, *latency*, and the *time* to peak amplitude (demonstrated in Fig. 6). The peak amplitude is the measure of the absolute largest displacement of force from zero in grams (between *B* and *C*). The latency to peak amplitude is measured from the onset of the stimulus to the force crossing the 50 g threshold (between *O* and *A*), and time to peak is measured from the onset of the stimulus to the time of peak amplitude (between *O* and *C*). The latency and time are recorded in seconds.

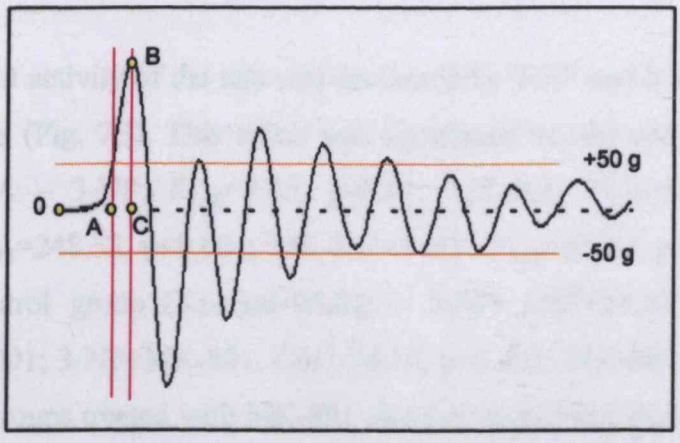

Figure 6 Typical example of a startle response waveform with the specific measuring points. See text for details.

During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed.

2.3. Statistical analysis

The number of noise-positive responses in the ASR and PPI test was evaluated by Chi^2 test. Distribution of OF, rotarod and other ASR/PPI data was checked for normality by Kolmogorov-Smirnov test. In case of normal distribution, all behavioural data were tested by one-way analysis of variance (ANOVA) (*post hoc* Scheffe test) providing *F* and *p* values. At non-normal distribution, by Kruskal-Wallis (*post hoc* Mann-Whitney test) was used, providing Chi^2 and *p* values. Significance was accepted at $p<0.05$ in all tests. The software pack SPSS 9.0 was used to the statistical analysis.

3. Results

3.1. Immediate effects

3.1.1. Spontaneous locomotor activity

A single dose of 3-NP caused significant decrease in the rats' ambulatory activity (ANOVA: $F_{1,18}$=17.13, p<0.001), compared to the pre-administration self-control (Fig. 7A). The effect of MK-801 was similar ($F_{1,18}$=5.10, p<0.05). The diminishing effect of 3-NP on the ambulation remained observable when it was given after MK-801 in the combination group. The effect of the opposite combination (3-NP+MK-801) was not significant.

The vertical activity of the rats was decreased by 3-NP and MK-801 alone, and by both combinations (Fig. 7B). This effect was significant vs. the pre-administration self-controls (ANOVA – 3-NP: $F_{1,18}$=9.15, p<0.01; MK-801: $F_{1,18}$=136.88, p<0.001; 3-NP+MK-801: $F_{1,18}$=248.32, p<0.001; MK-801+3-NP: $F_{1,18}$=66.24, p<0.001), and vs. the saline-treated control group (Kruskal-Wallis – 3-NP: Chi^2=34.10, p<0.01; MK-801: Chi^2=34.10, p<0.001; 3-NP+MK-801: Chi^2=34.10, p<0.001; MK-801+3-NP: Chi^2=34.10, p<0.001). In the groups treated with MK-801 alone or combined, significant (Chi^2=34.10, p<0.05) decrease of the vertical activity was also seen on comparison to the 3-NP treated group.

Administered alone, 3-NP ($F_{1,18}$=15.27, p<0.01) or MK-801 ($F_{1,18}$=10.45, p<0.01) caused significant local hyperactivity. A trend of increased local motility was also observed in the combination groups but with no significance (Fig. 7C).

When given to the rats before 3-NP administration, both QP ($F_{3,36}$=9.757, p<0.01) and SP ($F_{3,36}$=9.757, p<0.001) caused further decrease of ambulatory activity (Fig. 8A), so that the difference became significant vs. the saline-treated control group (QP: $F_{3,36}$=9.757, p<0.01; SP: $F_{3,36}$=9.757, p<0.001). It is noteworthy that the absolute values of activity (ambulatory, vertical and local) were highly similar in the control and 3-NP only groups in this and in the previous (3-NP combined with MK-801) experiment, indicating the stability of the data used for comparison and the correctness of comparison between individual experiments. On the vertical activity, the effect of QP+3-NP and

SP+3-NP was similar (Fig. 8B), except that here the decrease was significant both vs. control (QP+3-NP: Chi^2=23.81, p<0.001; SP+3-NP: Chi^2=23.81, p<0.001) and 3-NP groups (QP+3-NP: Chi^2=23.81, p<0.05; SP+3-NP: Chi^2=23.81, p<0.05). Local (grooming) activity was not altered by 3-NP (Fig. 8C). Compared to that, QP+3-NP caused an increase which was significant vs. control ($F_{3,36}$=43.718, p<0.001) and vs. 3-NP ($F_{3,36}$=43.718, p<0.001). SP, on the contrary, had no influence on the local activity.

In another series, QP and SP were combined with MK-801 (Fig. 9). The direction of changes induced by MK-801 was similar to those shown in Fig. 7 but, this time, only the decrease of vertical activity was significant vs. control (Chi^2=24.9, p<0.001). This massive effect (Fig. 9B) was somewhat strengthened by QP and SP but not significantly.

As described in methods (2.2.1), the four forms of OF activity (ambulatory, vertical, local, immobility) completely filled the length of an OF session. Table II shows the proportions of these forms of activity in the groups treated with 3-NP and with MK-801. The data show that the decrease in ambulation and rearing was paralleled by an increase of local activity and immobility.

Table II The proportions of ambulatory, vertical and local activity, and immobility time within the total open field activity before and after 3-NP (20 mg/kg) and MK-801 (0.8 mg/kg) administration in the immediate scheme.

	Proportion of the forms of OF activity	
3-NP alone (20 mg/kg)	Before treatment	30 min after treatment
Ambulatory activity	54.28%	28.11%
Vertical activity	12.81%	6.17%
Local activity	26.82%	37.94%
Immobility	6.09%	27.78%
MK-801 alone (0.8 mg/kg)	Before treatment	30 min after treatment
Ambulatory activity	52.23%	28.54%
Vertical activity	16.47%	0.43%
Local activity	26.44%	52.71%
Immobility	4.86%	18.32%

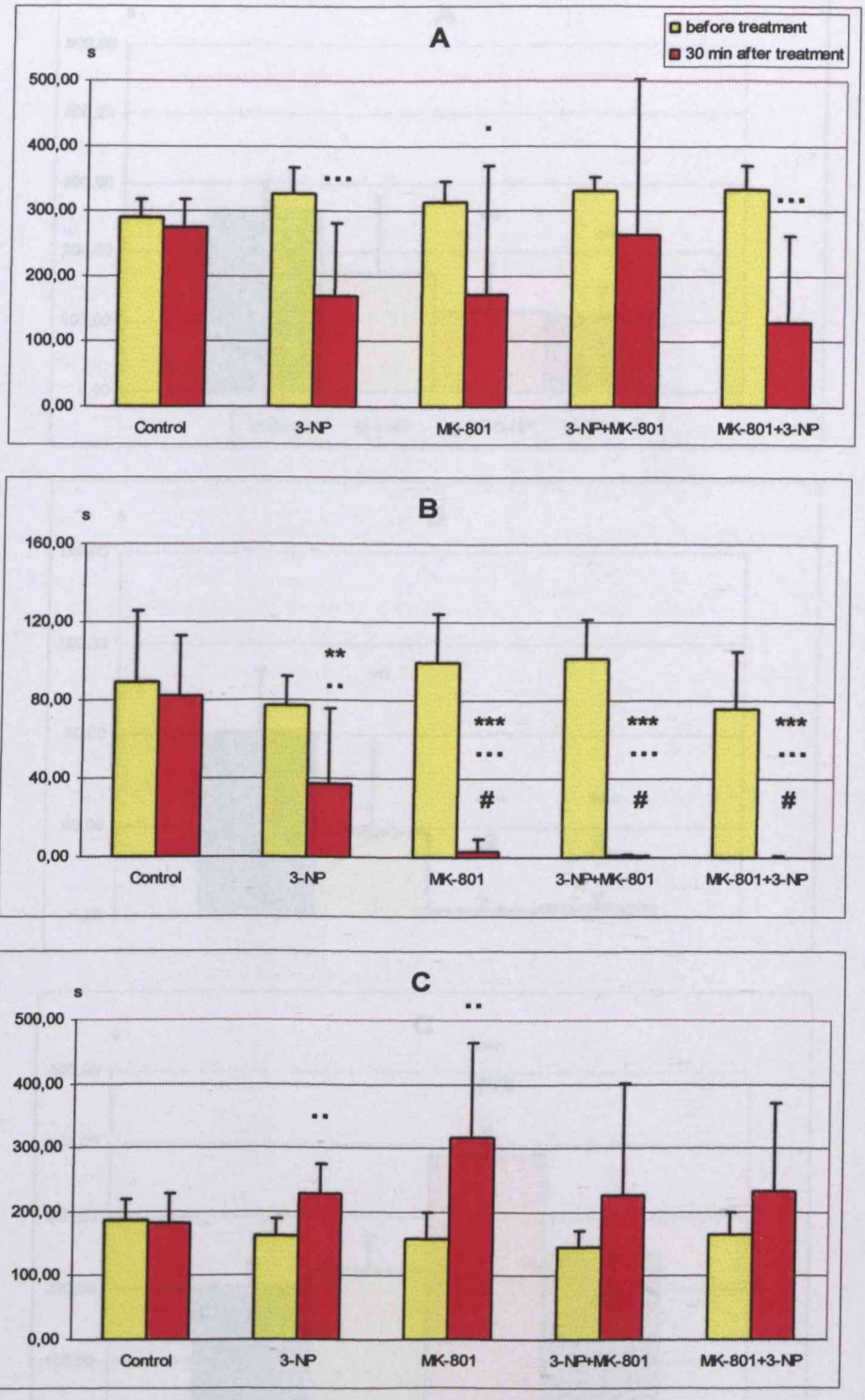

Figure 7 Spontaneous ambulatory (**A**), vertical (**B**), and local (**C**) exploratory activity of the rats in the open field box. In each (altogether 5) group, the left column represents the values before, and the right column, 30 min after, the (saline, 3-NP, MK-801, 3-NP+MK-801, MK-801+3-NP) treatments indicated on the abscissa. Ordinate: time (s) spent in the given forms of activity, mean+S.D.
** $p<0.01$, *** $p<0.001$ vs. saline-treated control group; ▪ $p<0.05$, ▪▪ $p<0.01$, ▪▪▪ $p<0.001$ vs. pre-administration data (self-control); # $p<0.05$ vs. 3-NP-treated group.

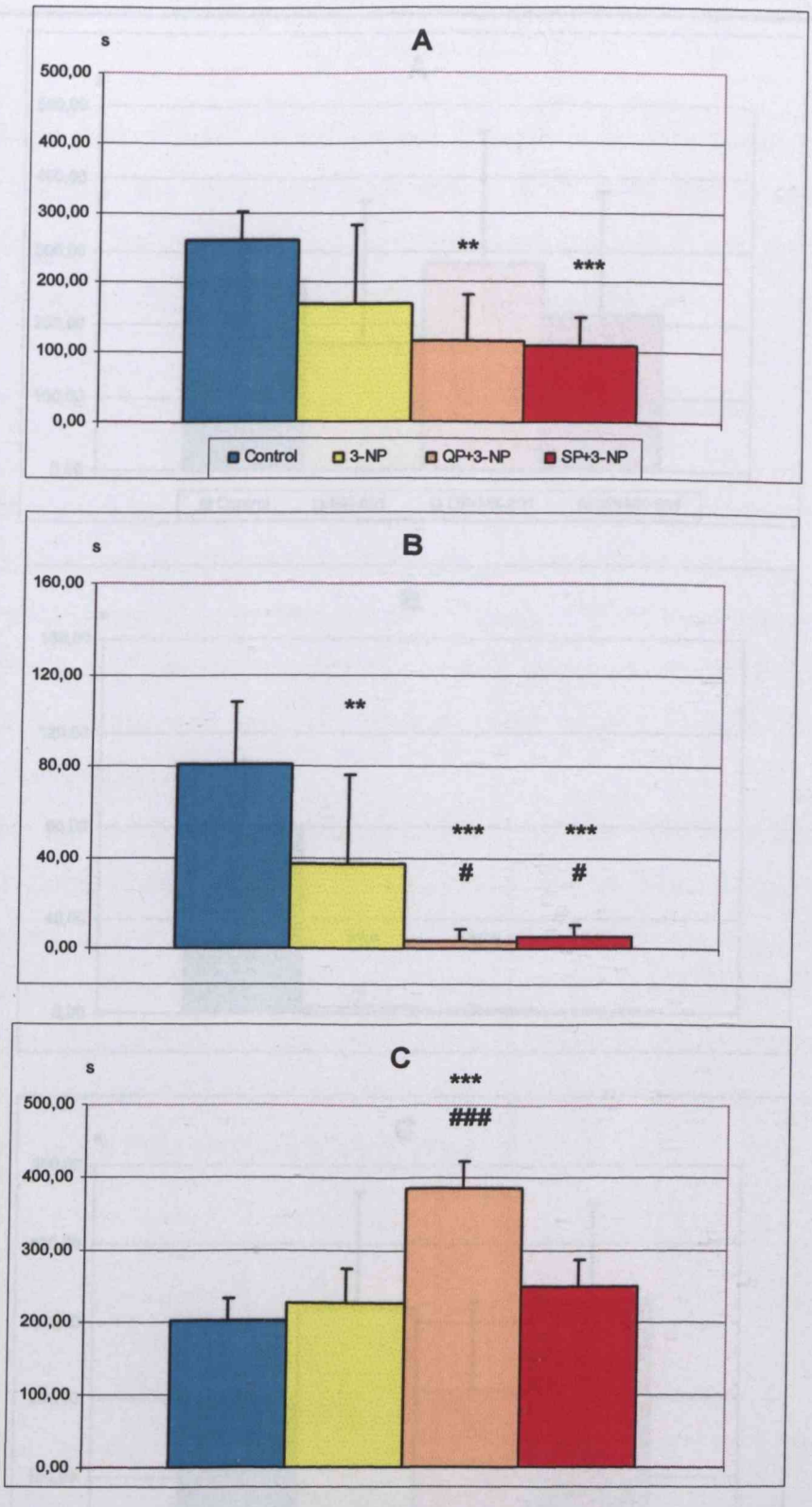

Figure 8 Spontaneous ambulatory (**A**), vertical (**B**), and local (**C**) exploratory activity of the rats in the open field box 30 min after the treatments indicated in the insert in **A**. Ordinate: time (s) spent in the given forms of activity, mean+S.D.
** $p<0.01$, *** $p<0.001$ vs. saline-treated control group; # $p<0.05$, ### $p<0.001$ vs. 3-NP-treated group.

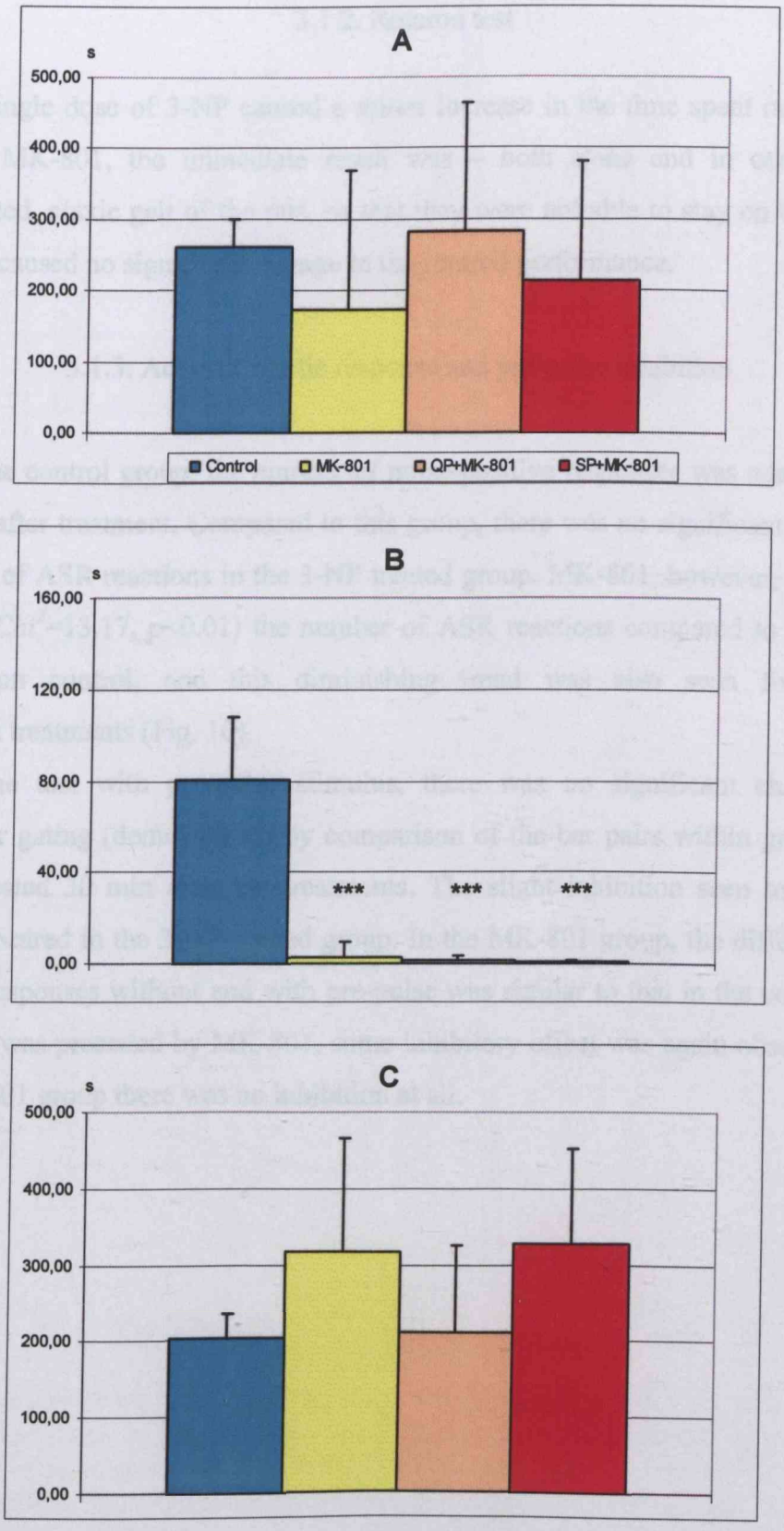

Figure 9 Spontaneous ambulatory (**A**), vertical (**B**), and local (**C**) exploratory activity of the rats in the open field box 30 min after the treatments. Displayed as in Fig. 8. *** $p<0.001$ vs. saline-treated control group.

3.1.2. Rotarod test

A single dose of 3-NP caused a minor increase in the time spent on the rotating rod. With MK-801, the immediate result was – both alone and in combinations – uncoordinated, ataxic gait of the rats, so that they were not able to stay on the rod at all. QP and SP caused no significant change in the rotarod performance.

3.1.3. Acoustic startle response and pre-pulse inhibition

In the control group, the number of noise-positive responses was nearly the same before and after treatment. Compared to this group, there was no significant alteration in the number of ASR reactions in the 3-NP treated group. MK-801, however, significantly decreased (Chi^2=13.17, p<0.01) the number of ASR reactions compared to its own pre-administration control, and this diminishing trend was also seen following the combination treatments (Fig. 10).

In the test with pre-pulse stimulus, there was no significant change in the sensorimotor gating (demonstrated by comparison of the bar pairs within groups in Fig. 11) when tested 30 min after the treatments. The slight inhibition seen in the control group disappeared in the 3-NP treated group. In the MK-801 group, the difference in the number of responses without and with pre-pulse was similar to that in the control group. When 3-NP was preceded by MK-801, some inhibitory effect was again observed, but in 3-NP+MK-801 group there was no inhibition at all.

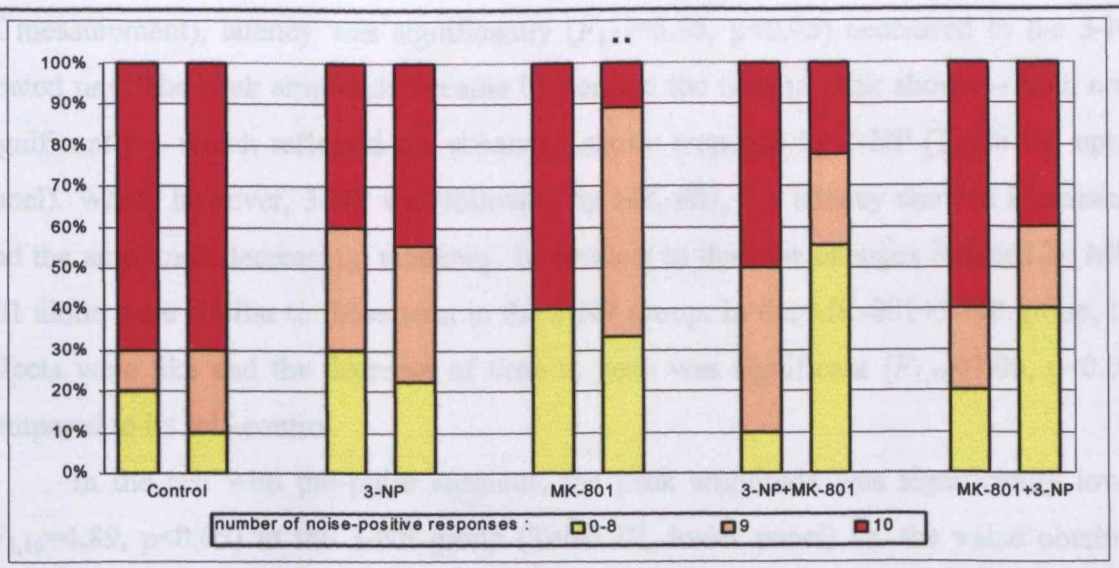

Figure 10 Number of noise-positive responses in the ASR test. In each group, the left column represents the distribution of responses before, and the right column, 30 min after the treatments indicated on the abscissa. Ordinate: percentage of the animals giving as many noise-positive responses as indicated in the insert.

** $p<0.01$ vs. self-control group.

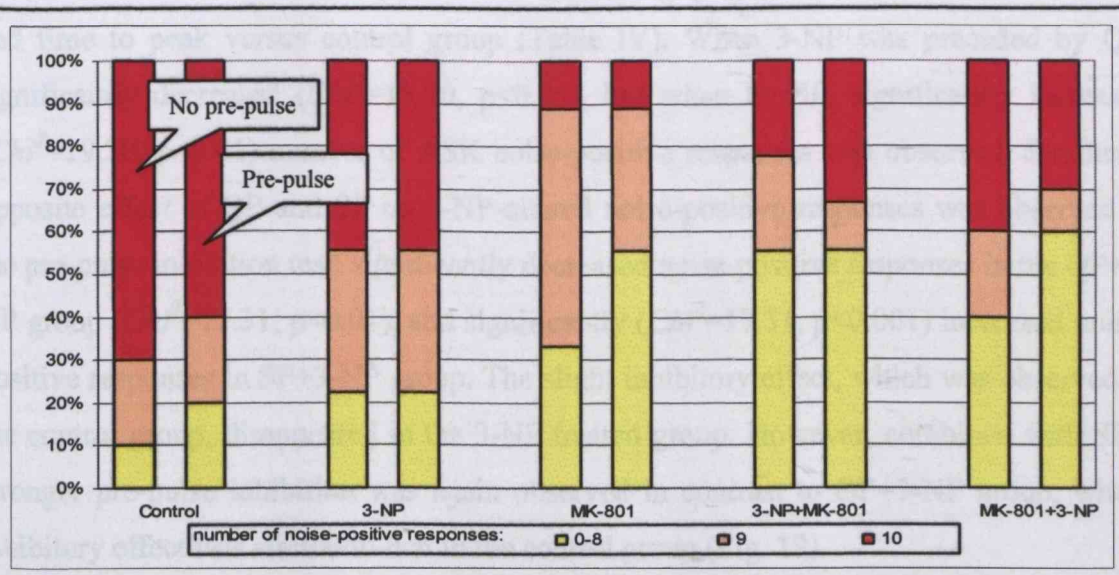

Figure 11 Number of noise-positive responses in the ASR test, 30 min after the treatments indicated on the abscissa. In each group, the left column represents the distribution of responses without (ASR), and the right column with (PPI), pre-pulse stimulus. Ordinate: percentage of the animals giving as many noise-positive responses as indicated in the insert.

Of the measured numeric parameters of the ASR response (see Fig. 6. for the way of measurement), latency was significantly ($F_{1,17}$=5.95, p<0.05) decreased in the 3-NP treated rats. The peak amplitude became higher and the time to peak shorter – both non-significantly – which reflected the enhanced startle response by 3-NP (Table III, upper panel). When, however, 3-NP was followed by MK-801, the latency showed increasing, and the amplitude decreasing, tendency. In contrast to that, the changes induced by MK-801 alone were similar to those seen in the 3-NP group. In the MK-801+3-NP group, the effects were like and the decrease of time to peak was significant ($F_{1,18}$=7.00, p<0.05) compared to its self-control.

In the test with pre-pulse stimulus, the peak amplitude was significantly lower ($F_{1,16}$=4.89, p<0.05) in the 3-NP group (Table III, lower panel) vs. the value obtained without pre-pulse (that is, the inhibitory effect of the pre-pulse was detectable on this parameter). A similar decreasing trend was observed in both combination groups, but that was not significant. MK-801 significantly ($F_{1,17}$=5.28, p<0.05) increased the time to peak versus self-control, which remained in the combinations groups.

In another series of the experiment, combinations with QP and SP, 3-NP again decreased the number of noise-positive responses (Fig. 12), and caused shorter latency and time to peak versus control group (Table IV). When 3-NP was preceded by QP, significantly decreased (Chi^2=19.20, p<0.05), but when by SP, significantly increased (Chi^2=19.20, p<0.01) number of ASR noise-positive responses was observed. Similarly, opposite effect of QP and SP on 3-NP-altered noise-positive responses was observed in the pre-pulse inhibition test: significantly decreased noise-positive responses in the QP+3-NP group (Chi^2=17.31, p<0.01), and significantly (Chi^2=17.31, p<0.001) increased noise-positive responses in SP+3-NP group. The slight inhibitory effect, which was observed in the control group, disappeared in the 3-NP treated group. However, combined with SP a stronger pre-pulse inhibition was again observed in contrast to QP+3-NP group, where inhibitory effect was similar to that in the control group (Fig. 12).

Combined with MK-801 significant contrary effects of QP and SP on MK-801 altered noise-positive responses were not recorded. Compared to the control, QP+MK-801 treatment significantly decreased the number of noise-positive responses both in absence (Chi^2=14.42, p<0.01) and presence (Chi^2=11.38, p<0.01) of the pre-pulse. There

was a minor, non-significant change in pre-pulse inhibition in MK-801 alone group, which was altered only moderately by QP or SP (Fig. 13).

Table III Measured numeric parameters (latency, peak amplitude, time to peak) of the noise-positive acoustic startle responses before and 30 min after the treatments, without (ASR, upper panel) and with (PPI, lower panel) pre-pulse stimulus. Mean±S.D.

	Treatments				
ASR	**Control** (n=10)	**3-NP** (n=10)	**MK-801** (n=10)	**3-NP +MK-801** (n=10)	**MK-801 +3-NP** (n=10)
Latency (ms)					
before treatment	18.4±1.95	18.70±2.15	18.39±1.98	18.18±1.73	18.64±1.83
after treatment	16.8±1.78	16.68±1.31 ▪	17.33±1.53	18.90±2.55	17.23±1.11
Peak amplitude (g)					
before treatment	312.6±144.0	276.3±131.2	330.9±170.3	268.3±137.8	255.1±79.80
after treatment	447.8±201.0	355.8±102.1	409.4±148.2	221.8±65.60	333.0±150.0
Time to peak (ms)					
before treatment	25.85±1.34	24.85±1.79	24.73±1.40	24.91±0.90	25.64±1.72
after treatment	24.93±0.95	24.50±0.75	25.19±1.38	24.93±2.00	24.07±0.74 ▪

	Treatments				
PPI	**Control** (n=10)	**3-NP** (n=10)	**MK-801** (n=10)	**3-NP +MK-801** (n=10)	**MK-801 +3-NP** (n=10)
Latency (ms)					
before treatment	17.43±1.75	19.29±2.81	17.4±1.67	18.14±1.73	17.6±1.77
after treatment	17.08±2.37	17.93±1.67	17.7±0.98	19.03±2.92	18.32±2.02
Peak amplitude (g)					
before treatment	303.1±192.6	206.9±90.18	280.8±105.1	223.3±84.5	270.9±129.3
after treatment	385.0±203.5	246.9±106.7 ¤	362.0±162.5	206.2±106.2	252.3±99.4
Time to peak (ms)					
before treatment	23.89±0.85	25.01±1.48	23.91±1.05	23.34±0.95	24.03±1.23
after treatment	24.16±1.35	24.44±0.82	24.93±0.85 ▪	24.55±1.91	24.13±1.56

n = number of animals in the groups.

▪ $p<0.05$ vs. "before treatment" data of the same group (self-control); ¤ $p<0.05$ "after treatment" data, with vs. without pre-pulse.

was a minor, non-significant change in pre-pulse inhibition in MK-801 alone group, which was altered only moderately by QP or SP (Fig. 13).

Table III Measured numeric parameters (latency, peak amplitude, time to peak) of the noise-positive acoustic startle responses before and 30 min after the treatments, without (ASR, upper panel) and with (PPI, lower panel) pre-pulse stimulus. Mean±S.D.

	Treatments				
ASR	**Control** (n=10)	**3-NP** (n=10)	**MK-801** (n=10)	**3-NP +MK-801** (n=10)	**MK-801 +3-NP** (n=10)
Latency (ms)					
before treatment	18.4±1.95	18.70±2.15	18.39±1.98	18.18±1.73	18.64±1.83
after treatment	16.8±1.78	16.68±1.31 ▪	17.33±1.53	18.90±2.55	17.23±1.11
Peak amplitude (g)					
before treatment	312.6±144.0	276.3±131.2	330.9±170.3	268.3±137.8	255.1±79.80
after treatment	447.8±201.0	355.8±102.1	409.4±148.2	221.8±65.60	333.0±150.0
Time to peak (ms)					
before treatment	25.85±1.34	24.85±1.79	24.73±1.40	24.91±0.90	25.64±1.72
after treatment	24.93±0.95	24.50±0.75	25.19±1.38	24.93±2.00	24.07±0.74 ▪

	Treatments				
PPI	**Control** (n=10)	**3-NP** (n=10)	**MK-801** (n=10)	**3-NP +MK-801** (n=10)	**MK-801 +3-NP** (n=10)
Latency (ms)					
before treatment	17.43±1.75	19.29±2.81	17.4±1.67	18.14±1.73	17.6±1.77
after treatment	17.08±2.37	17.93±1.67	17.7±0.98	19.03±2.92	18.32±2.02
Peak amplitude (g)					
before treatment	303.1±192.6	206.9±90.18	280.8±105.1	223.3±84.5	270.9±129.3
after treatment	385.0±203.5	246.9±106.7 ¤	362.0±162.5	206.2±106.2	252.3±99.4
Time to peak (ms)					
before treatment	23.89±0.85	25.01±1.48	23.91±1.05	23.34±0.95	24.03±1.23
after treatment	24.16±1.35	24.44±0.82	24.93±0.85 ▪	24.55±1.91	24.13±1.56

n = number of animals in the groups.

▪ $p<0.05$ vs. "before treatment" data of the same group (self-control); ¤ $p<0.05$ "after treatment" data, with vs. without pre-pulse.

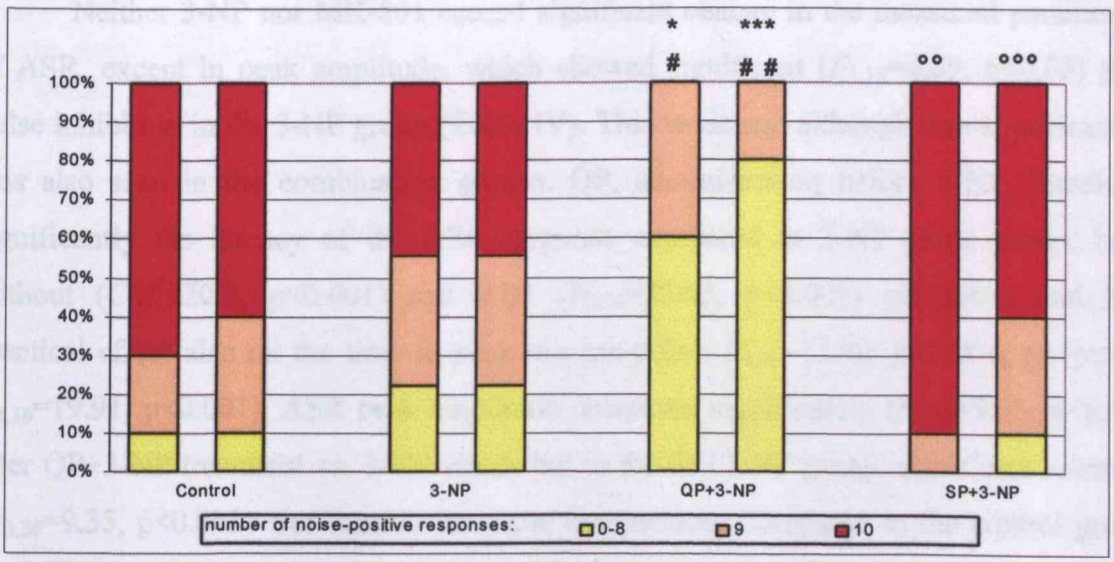

Figure 12 Number of noise-positive responses in the ASR test, 30 min after the treatments indicated on the abscissa. Displayed as in Fig. 11.
* $p<0.05$, *** $p<0.001$ vs. saline-treated control group; # $p<0.05$, ## $p<0.01$ vs. 3-NP-treated group; °° $p<0.01$, °°° $p<0.001$ vs. QP+3-NP group.

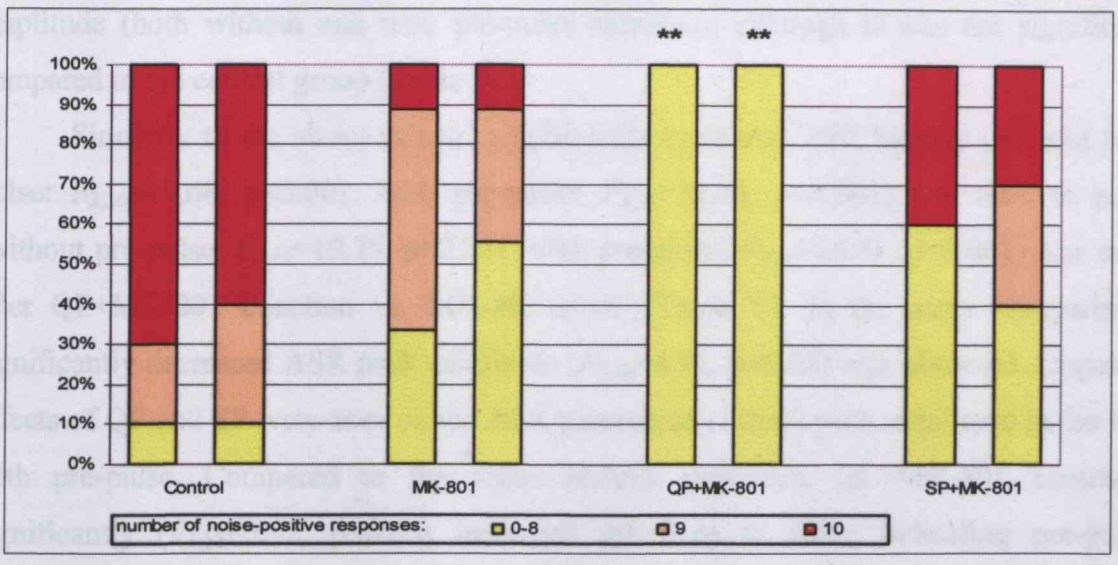

Figure 13 Number of noise-positive responses in the ASR test, 30 min after the treatments indicated on the abscissa. Displayed as in Fig. 11.
** $p<0.01$ vs. saline-treated control group.

Neither 3-NP nor MK-801 caused significant change in the measured parameters of ASR, except in peak amplitude, which showed significant ($F_{1,16}$=4.89, p<0.05) pre-pulse inhibition in the 3-NP group (Table IV). This tendency, although non-significantly, was also seen in the combination groups. QP, administration before 3-NP, increased significantly the latency of the ASR response compared to 3-NP alone group, both without (Chi^2=20.8, p<0.001) and with ($F_{3,36}$=25.63, p<0.001) pre-pulse; and had identical effect also on the time to peak (no pre-pulse: $F_{3,36}$=17.40, p<0.001; pre-pulse: $F_{3,36}$=19.91, p<0.001). ASR peak amplitude decreased significantly ($F_{3,36}$=9.35, p<0.05) after QP+3-NP treatment vs. 3-NP alone; but in the SP+3-NP group, significant increase ($F_{3,36}$=9.35, p<0.001) was seen in the same comparison. Compared to the control group similar changes was seen after QP+3-NP treatment: significantly increased latency (without pre-pulse: Chi^2=20.8, p<0.001; with pre-pulse: $F_{3,36}$=25.63, p<0.001) and time to peak (without pre-pulse: $F_{3,36}$=17.40, p<0.001; with pre-pulse: $F_{3,36}$=19.91, p<0.001), and decreased peak amplitude (without pre-pulse: $F_{3,36}$=9.35, p<0.01; with pre-pulse: $F_{3,36}$=7.94, p<0.01). Opposite effect of QP and SP was also seen in latency and peak amplitude (both without and with pre-pulse stimulus), although it was not significant compared to the control group (Table IV).

Similarly to the above effects, significantly increased ASR latency (without pre-pulse: $F_{3,36}$=19.68, p<0.001, with pre-pulse: $F_{3,36}$=22.96, p<0.001) and time to peak (without pre-pulse: $F_{3,36}$=19.75, p<0.001; with pre-pulse: $F_{3,36}$=16.41, p<0.001) was seen after QP+MK-801 injection vs. MK-801 alone (Table V). In the same comparison, significantly decreased ASR peak amplitude ($F_{3,36}$=4.91, p<0.05) was observed. Opposite effects of QP and SP were seen on the ASR parameters, except peak amplitude in the test with pre-pulse. Compared to the value without pre-pulse, QP+MK-801 treatment significantly ($F_{1,13}$=5.92, p<0.05) increased the time to peak, indicating pre-pulse inhibition and reversing the slight decrease of this parameter seen in the MK-801 only group. Compared to the control group similar changes was detectable after QP+MK-801 treatment, which was seen comparing to MK-801 alone group (Table V).

Table IV Measured parameters (latency, peak amplitude, time to peak) of the acoustic startle responses without (ASR) or with (PPI) pre-pulse stimulus 30 min after the treatments. Mean±S.D.

	Treatments			
	Control (n=10)	**3-NP** (n=10)	**QP+3-NP** (n=10)	**SP+3-NP** (n=10)
ASR				
Latency (ms)	17.17±1.06	16.68±1.31	21.82±2.56 ***###	16.89±0.83 °°°
Peak amplitude (g)	383.2±147.7	355.8±102.1	191.8±75.39 **#	459.3±126.6 °°°
Time to peak (ms)	24.54±1.00	24.50±0.75	28.60±1.9 ***###	25.24±1.80 °°°
PPI				
Latency (ms)	17.20±0.93	17.93±1.67	22.63±1.83 ***###	17.89±1.47 °°°
Peak amplitude (g)	349.2±129.7	246.9±106.7 ¤	136.0±56.23 **	355.4±133.9 °°
Time to peak (ms)	24.19±1.11	24.44±0.82	27.83±1.63 ***###	25.38±0.78 °°

n = number of animals in the groups.
** p<0.01, *** p<0.001 vs. saline-treated control group; # p<0.05, ### p<0.001 vs. 3-NP-treated group; °° p<0.01, °°° p<0.001 vs. QP+3-NP group; ¤ p<0.05 vs. without pre-pulse stimulus.

Table V Measured parameters (latency, peak amplitude, time to peak) of the acoustic startle responses without (ASR) or with (PPI) pre-pulse stimulus 30 min after the treatments. Mean±S.D.

	Treatments			
	Control (n=10)	**MK-801** (n=10)	**QP+MK-801** (n=10)	**SP+MK-801** (n=10)
ASR				
Latency (ms)	17.17±1.06	17.33±1.53	22.46±2.43 ***###	16.98±1.99 °°°
Peak amplitude (g)	383.2±147.7	409.4±148.2	176.3±95.7 #	418.5±208.0 °
Time to peak (ms)	24.54±1.00	25.19±1.38	28.07±1.34 ***###	24.25±1.07 °°°
PPI				
Latency (ms)	17.20±0.93	17.70±0.98	23.57±2.72 ***###	17.55±1.86 °°°
Peak amplitude (g)	349.2±129.7	362.0±162.5	168.1±82.63	325.1±223.7
Time to peak (ms)	24.19±1.11	24.93±0.85	29.74±124***###¤	24.40±1.06 °°°

n = number of animals in the groups.
*** p<0.001 vs. saline-treated control group; # p<0.05, ### p<0.001 vs. MK-801-treated group; ° p<0.05, °°° p<0.001 vs. QP+MK-801 group; ¤ p<0.05 vs. without pre-pulse stimulus.

3.2. Acute effects

3.2.1. Spontaneous locomotor activity

A single dose of 3-NP caused significant ($F_{1,18}$=16.95, p<0.001) decrease of the spontaneous ambulatory activity, compared to the pre-administration controls, also in this experimental scheme (Fig. 14A). Given alone, MK-801 had a similar, significant ($F_{1,18}$=20.37, p<0.001) effect. The 3-NP+MK-801 combination had a similar effect which was also significant ($F_{1,18}$=8.56, p<0.01), but when 3-NP was preceded by MK-801, there was no significant change in the ambulatory activity.

In the group treated with 3-NP only, an increase of rearing activity was seen 24 h after administration (Fig. 14B). A similar effect of MK-801 was significant ($F_{1,18}$=7.12, p<0.05). In contrast, spontaneous vertical hypoactivity was observed in the combination groups; this alteration, however, was significant ($F_{1,18}$=7.55, p<0.05) only in 3-NP+MK-801 group.

There was only a minor change in local motility in the groups treated with 3-NP and MK-801 alone (Fig. 14C). The 3-NP+MK-801 combined injection caused, however, significantly ($F_{1,18}$=10.53, p<0.01) increased spontaneous local hyperactivity, in contrast to the MK-801+3-NP combination group where the change was minimal.

Combined with DA modulators, there was no significant change in open field activity compared to the 3-NP alone group. But on comparison to control, SP+3-NP treatment caused significant ($F_{3,36}$=3.46, p<0.05) decrease of vertical activity (Fig. 15).

In another series, combining MK-801 with DA modulators, only ambulatory activity was reduced significantly ($F_{3,36}$=4.17, p<0.05) compared to MK-801 alone group in the SP+MK-801 treated rats. A minor, negligible change was observed in rearing and grooming by QP and SP (Fig. 16).

Similar to the effects in the immediate scheme, both 3-NP and MK-801 decreased the total open field locomotor activity, but the decrease of ambulatory activity was less than it was 30 min after the treatment. Vertical activity increased – in contrast to the noteworthy decreasing tendency seen 30 min after administration – and only moderate increase was seen in the proportions of grooming and immobility (Table VI).

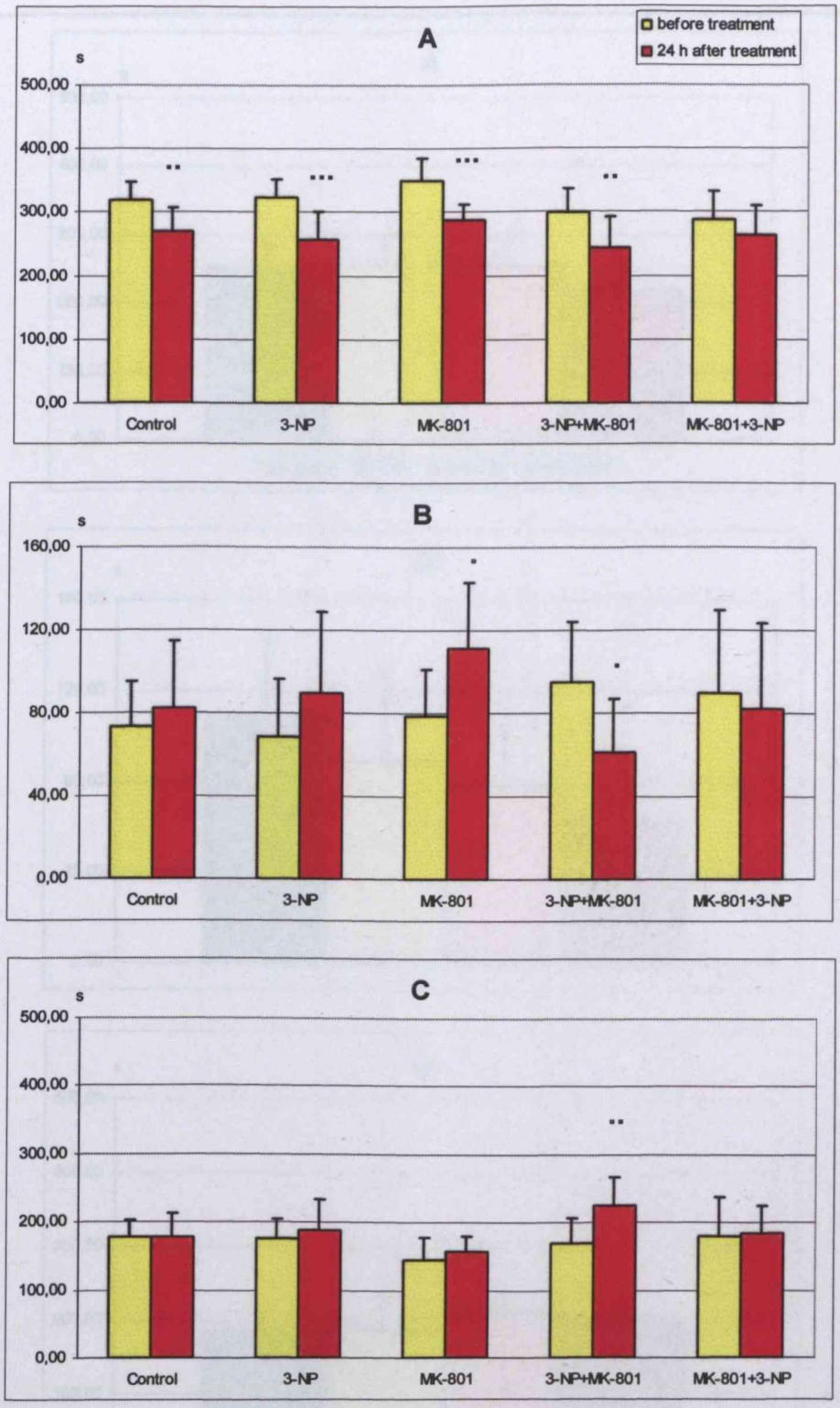

Figure 14 Spontaneous ambulatory (**A**), vertical (**B**), and local (**C**) exploratory activity of the rats in the open field box. In each (altogether 5) group, the left column represents the values before, and the right column, 24 h after, the (saline, 3-NP, MK-801, 3-NP+MK-801, MK-801+3-NP) treatments indicated on the abscissa.
Ordinate: time (s) spent in the given forms of activity, mean+S.D.
$^{\cdot}$ $p<0.05$, $^{\cdot\cdot}$ $p<0.01$, $^{\cdot\cdot\cdot}$ $p<0.001$ vs. pre-administration data (self-control).

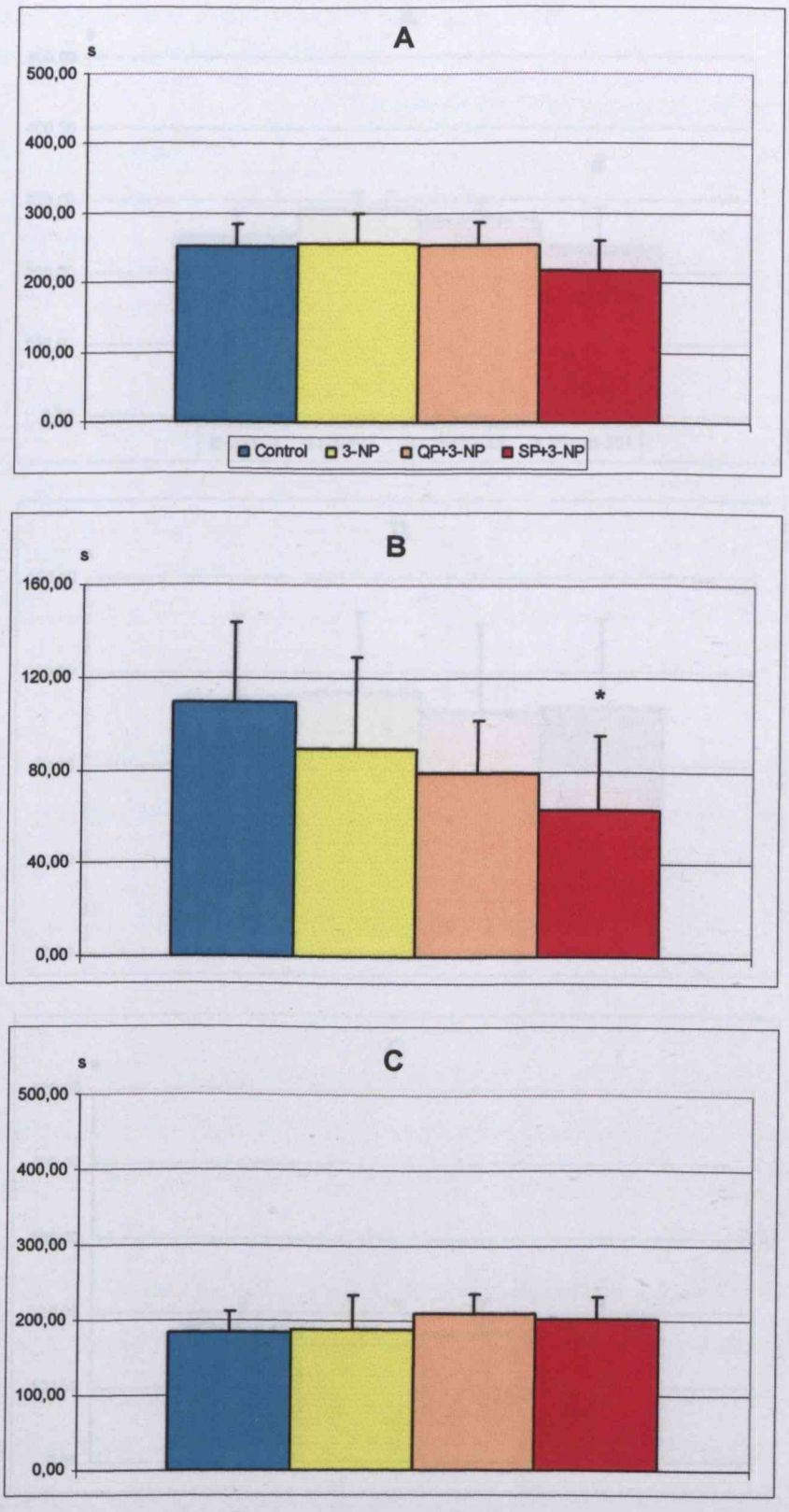

Figure 15 Spontaneous ambulatory (**A**), vertical (**B**), and local (**C**) exploratory activity of the rats in the open field box 24 h after the treatments indicated in the insert in **A**. Ordinate: time (s) spent in the given forms of activity, mean+S.D.
* $p<0.05$ vs. saline-treated control group.

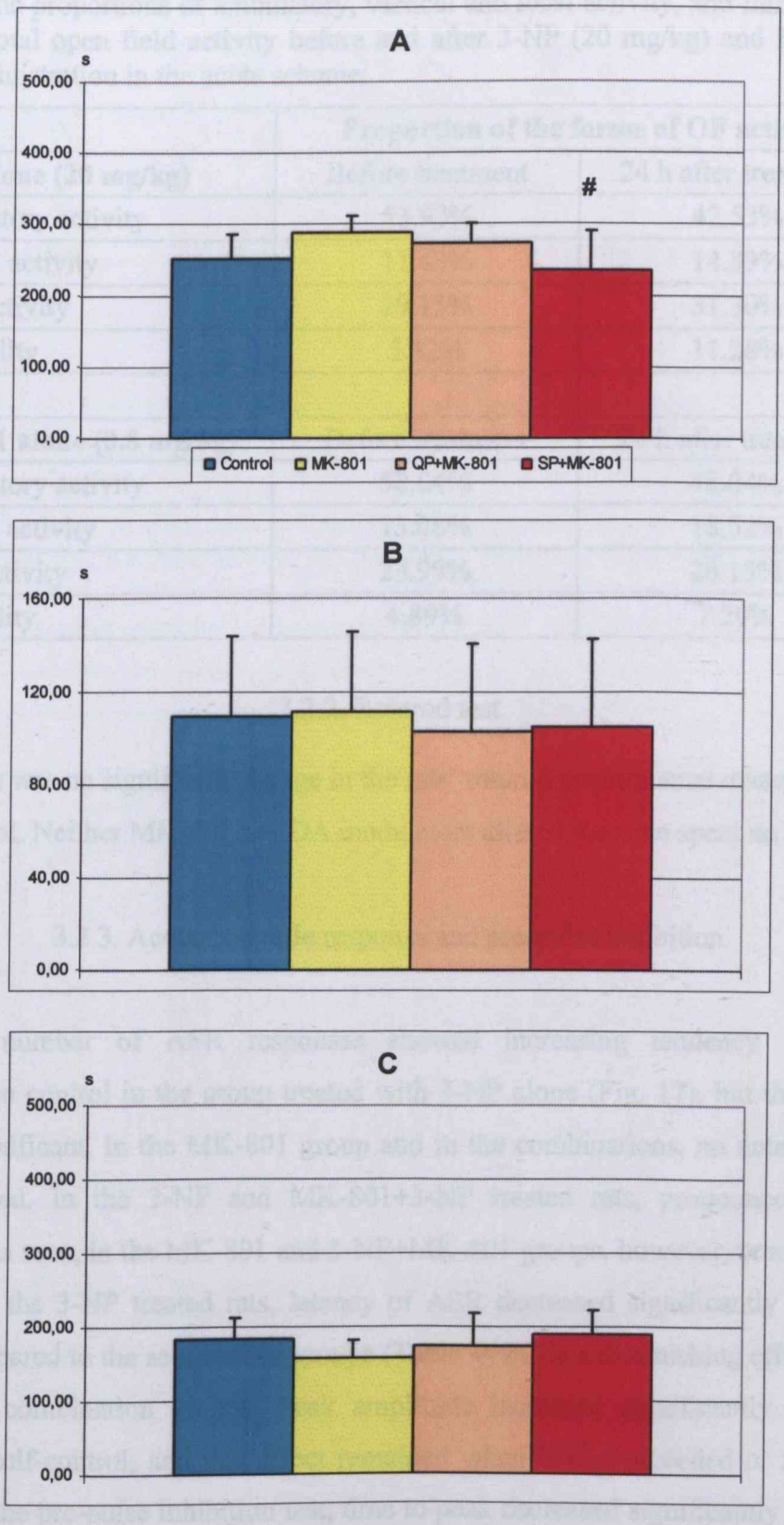

Figure 16 Spontaneous ambulatory (**A**), vertical (**B**), and local (**C**) exploratory activity of the rats in the open field box 24 h after the treatments. Displayed as in Fig. 15.
# $p<0.05$ vs. MK-801-treated group.

Table VI The proportions of ambulatory, vertical and local activity, and immobility time within the total open field activity before and after 3-NP (20 mg/kg) and MK-801 (0.8 mg/kg) administration in the acute scheme.

	Proportion of the forms of OF activity	
3-NP alone (20 mg/kg)	Before treatment	24 h after treatment
Ambulatory activity	53.90%	42.53%
Vertical activity	11.43%	14.89%
Local activity	29.15%	31.30%
Immobility	5.52%	11.28%
MK-801 alone (0.8 mg/kg)	Before treatment	24 h after treatment
Ambulatory activity	58.04%	48.04%
Vertical activity	13.08%	18.52%
Local activity	23.99%	26.15%
Immobility	4.89%	7.29%

3.2.2. Rotarod test

There was no significant change in the rats' rotarod performance treated with 3-NP vs. control. Neither MK-801 nor DA modulators altered the time spent on the rotarod.

3.2.3. Acoustic startle response and pre-pulse inhibition

The number of ASR responses showed increasing tendency versus pre-administration control in the group treated with 3-NP alone (Fig. 17), but the difference were not significant. In the MK-801 group and in the combinations, no notable changes were observed. In the 3-NP and MK-801+3-NP treated rats, pronounced pre-pulse inhibition was seen, in the MK-801 and 3-NP+MK-801 groups, however, practically none (Fig. 18). In the 3-NP treated rats, latency of ASR decreased significantly ($F_{1,18}$=8.53, $p<0.01$) compared to the self-control groups (Table VII). This diminishing effect was also seen in the combination groups. Peak amplitude increased significantly ($F_{1,18}$=6.65, $p<0.05$) vs. self-control, and this effect remained when it was preceded or followed by MK-801. In the pre-pulse inhibition test, time to peak decreased significantly ($F_{1,18}$=4.82, $p<0.05$) in the 3-NP alone group, which effect was also visible in the combined groups.

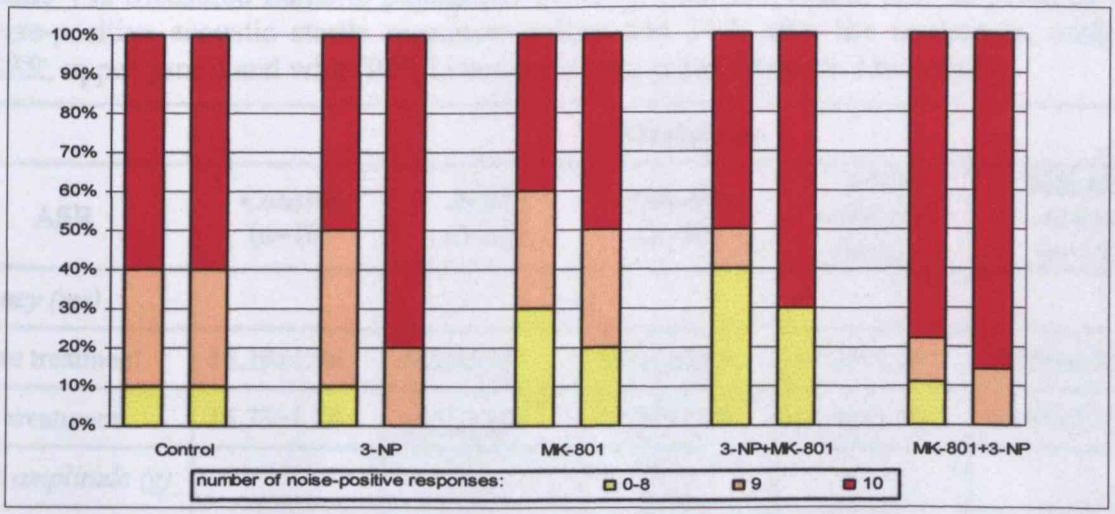

Figure 17 Number of noise-positive responses in the ASR test. In each group, the left column represents the distribution of responses before, and the right column, 24 h after the treatments indicated on the abscissa. Ordinate: percentage of the animals giving as many noise-positive responses as indicated in the insert.

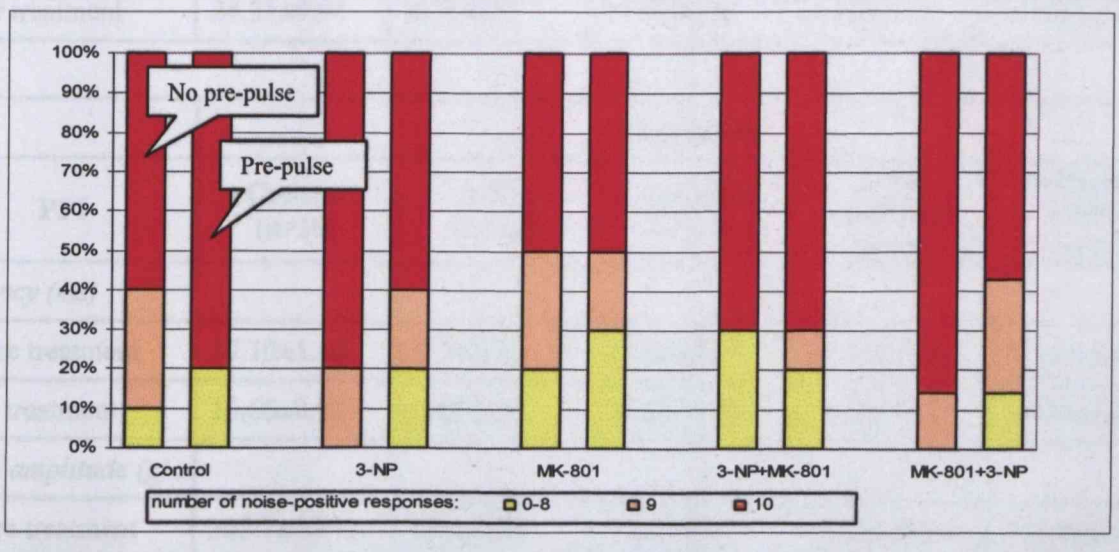

Figure 18 Number of noise-positive responses in the ASR test, 24 h after the treatments indicated on the abscissa. In each group, the left column represents the distribution of responses without (ASR), and the right column with (PPI), pre-pulse stimulus. Ordinate: percentage of the animals giving as many noise-positive responses as indicated in the insert.

In another series, the effects of 3-NP on the number of noise positive responses and on pre-pulse inhibition was minimally altered by QP or SP (Fig. 19). MK-801 induced minor change in the number of responses, which was also only slightly affected by QP and SP. Similar to noise-positive responses, pre-pulse inhibition was not altered by QP or SP (Fig. 20).

Table VII Measured numeric parameters (latency, peak amplitude, time to peak) of the noise-positive acoustic startle responses before and 24 h after the treatments, without (ASR, upper panel) and with (PPI, lower panel) pre-pulse stimulus. Mean±S.D.

	Treatments				
ASR	**Control** (n=10)	**3-NP** (n=10)	**MK-801** (n=10)	**3-NP +MK-801** (n=10)	**MK-801 +3-NP** (n=10)
Latency (ms)					
before treatment	18.19±1.96	18.55±2.30	18.07±2.63	17.92±1.69	18.29±2.27
after treatment	16.73±1.24	16.23±1.00 ▪▪	16.39±1.39	17.40±1.90	16.67±2.19
Peak amplitude (g)					
before treatment	319.9±125.3	301.5±104.2	377.6±202.0	242.2±127.1	297.4±131.8
after treatment	376.9±155.5	484.5±198.7 ▪	456.5±207.8	333.7±179.2	386.1±161.4
Time to peak (ms)					
before treatment	25.16±1.75	25.58±1.97	25.44±1.85	24.48±1.33	25.75±2.61
after treatment	24.31±0.94	25.76±2.57	24.60±1.30	24.56±1.17	24.52±1.54

	Treatments				
PPI	**Control** (n=10)	**3-NP** (n=10)	**MK-801** (n=10)	**3-NP +MK-801** (n=10)	**MK-801 +3-NP** (n=10)
Latency (ms)					
before treatment	17.10±1.10	17.24±1.92	17.59±2.47	17.70±2.18	17.43±1.48
after treatment	16.66±0.93	16.62±2.01	16.50±1.95	17.34±1.47	16.90±1.02
Peak amplitude (g)					
before treatment	305.7±96.43	339.3±163.6	372.0±287.4	247.6±130.4	271.0±124.7
after treatment	357.5±101.4	432.9±218.4	430.8±252.8	305.4±143.8	300.2±111.9
Time to peak (ms)					
before treatment	23.71±1.41	24.31±1.00	24.46±0.78	24.11±1.44	23.95±1.13
after treatment	23.80±0.71	23.84±1.04 ¤	23.83±0.78	24.53±0.68	23.70±0.77

n = number of animals in the groups.

▪ $p<0.05$, ▪▪ $p<0.01$ vs. "before treatment" data of the same group (self-control); ¤ $p<0.05$ "after treatment" data, with vs. without pre-pulse.

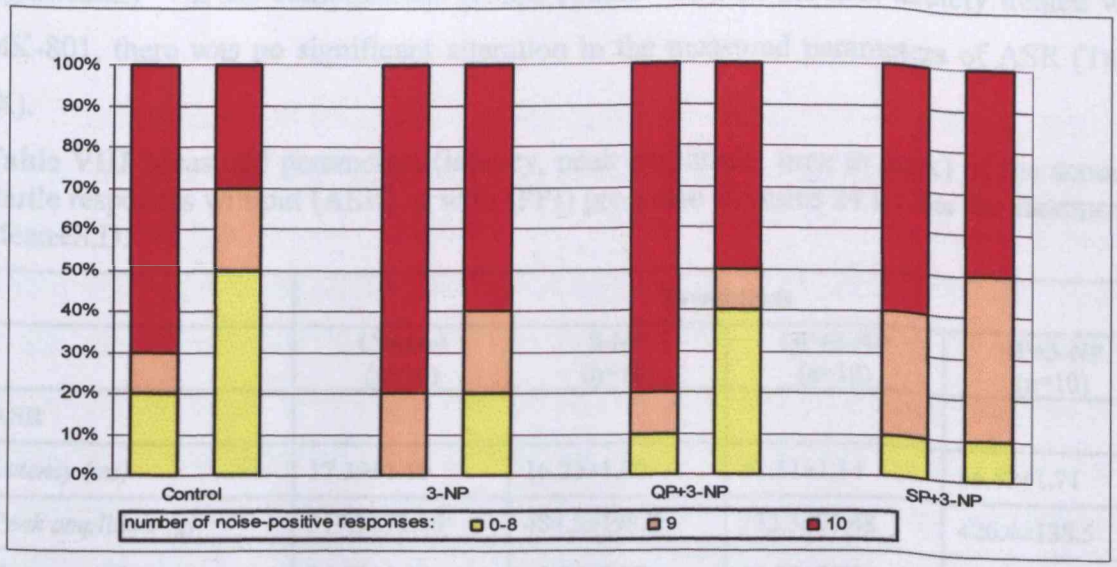

Figure 19 Number of noise-positive responses in the ASR test, 24 h after the treatments indicated on the abscissa. Displayed as in Fig. 18.

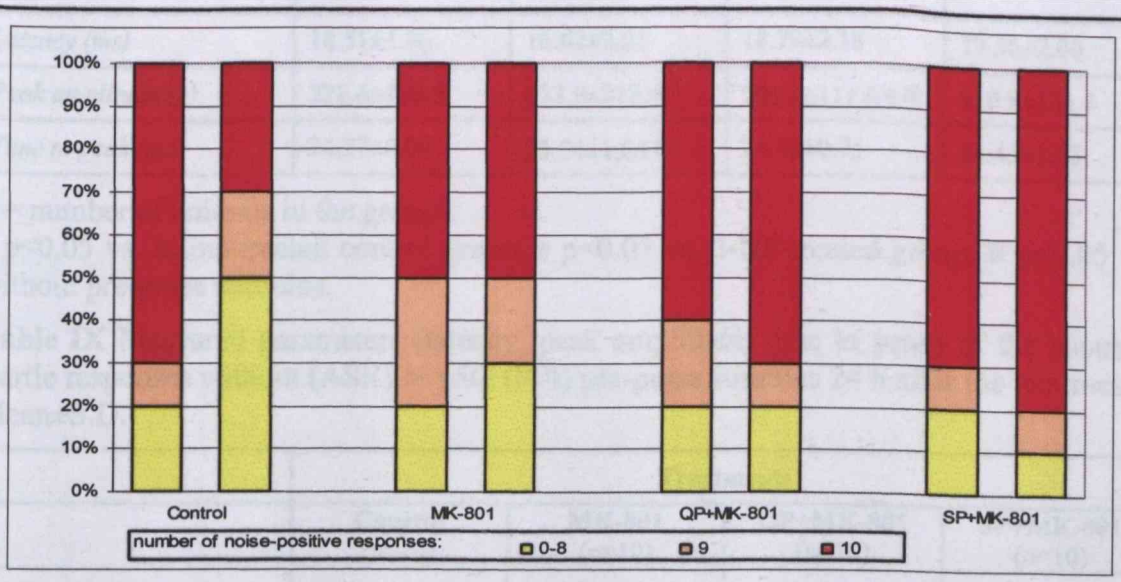

Figure 20 Number of noise-positive responses in the ASR test, 24 h after the treatments indicated on the abscissa. Displayed as in Fig. 18.

Of the measured parameters of ASR, 3-NP caused a significant ($F_{3,36}$=4.78, $p<0.05$) increase of peak amplitude in the test also here, which was decreased significantly in the QP+3-NP group compared to 3-NP alone ($F_{3,36}$=4.78, $p<0.05$). Peak amplitude decreased in 3-NP alone group compared to without pre-pulse data, this inhibitory effect of pre-pulse became significant ($F_{1,18}$=4.41, $p<0.05$), when 3-NP was preceded by QP. Pre-pulse inhibition was also seen in time to peak, which decreased significantly ($F_{1,18}$=4.41, $p<0.05$) in 3-NP treated group. This effect remained – but not

significantly – in the combinations groups (Table VIII). In the rats, acutely treated with MK-801, there was no significant alteration in the measured parameters of ASR (Table IX).

Table VIII Measured parameters (latency, peak amplitude, time to peak) of the acoustic startle responses without (ASR) or with (PPI) pre-pulse stimulus 24 h after the treatments. Mean±S.D.

	Treatments			
	Control (n=10)	**3-NP** (n=10)	**QP+3-NP** (n=10)	**SP+3-NP** (n=10)
ASR				
Latency (ms)	17.39±1.10	16.23±1.00	17.51±1.14	16.82±1.71
Peak amplitude (g)	319.3±99.06	484.5±198.7	332.3±97.48	426.4±138.5
Time to peak (ms)	24.72±0.83	25.76±2.57	24.67±0.80	24.72±1.46
PPI				
Latency (ms)	18.51±1.90	16.62±2.01	18.79±2.18	17.50±2.00
Peak amplitude (g)	228.6±108.0	432.9±218.4 *	205.4±111.6 # ¤	316.8±131.3
Time to peak (ms)	24.27±0.90	23.84±1.04 ¤	24.42±0.75	24.43±1.43

n = number of animals in the groups.
* $p<0.05$ vs. saline-treated control group; # $p<0.05$ vs. 3-NP-treated group; ¤ $p<0.05$ vs. without pre-pulse stimulus.

Table IX Measured parameters (latency, peak amplitude, time to peak) of the acoustic startle responses without (ASR) or with (PPI) pre-pulse stimulus 24 h after the treatments. Mean±S.D.

	Treatments			
	Control (n=10)	**MK-801** (n=10)	**QP+MK-801** (n=10)	**SP+MK-801** (n=10)
ASR				
Latency (ms)	17.39±1.10	16.39±1.39	17.63±1.46	16.67±1.86
Peak amplitude (g)	319.3±99.06	456.5±207.8	326.4±149.2	433.5±195.5
Time to peak (ms)	24.72±0.83	24.60±1.30	24.31±0.98	24.49±1.25
PPI				
Latency (ms)	18.51±1.90	16.50±1.95	17.64±1.88	16.65±2.17
Peak amplitude (g)	228.6±108.0	430.8±252.8	319.5±169.3	459.2±297.5
Time to peak (ms)	24.27±0.90	23.83±0.78	24.11±1.07	24.44±1.53

n = number of animals in the groups.

4. Discussion

The spontaneous locomotor activity of the rats was significantly altered by 3-NP, both 30 min and 24 h after administration. The general trend: decreased locomotion, and increased local activity and immobility, was consistent with the observation of Seaman (2000) who detected similar hypoactivity in rats systemically treated with 3-NP. In other studies, first hyper- then hypoactivity was observed following acute administration of 3-NP (Borlongan et al., 1997a); or pure hypoactivity in a 28 days study (Koutouzis et al., 1994), which was supposed to result from the lesions of the basal ganglia. The level of motor activity and DA concentration are closely related, as demonstrated by Miller et al. (2002), who detected higher levels of locomotion in *d*-amphetamine treated rats and this behaviour was associated with a significant enhancement of DA in the dorsal striatum. Such effect of *d*-amphetamine was described e.g. by Clarkson et al. (1988). The reduced motor behaviour in our study was thus consistent with some reports where intrastriatal injections of 3-NP reduced the striatal DA concentration, most likely via killing intrinsic striatal neurons (Beal et al., 1993; Brouillet et al., 1993). Vertical motility seems to be especially sensitive to the dopaminergic state of the striatum (Sedelis et al., 2001). In an animal model of Parkinson's disease, mice showed decreased rearing activity following administration of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) a drug selectively affecting dopaminergic neurons. So, the strongly reduced rearing in the rats treated with 3-NP was another indicator of its effect on DA concentration. 24 h after the administration of 3-NP the rate of decrease of the locomotor activity was less than in immediately treated rats. The concentration of 3-NP and its effect on dopaminergic system seemed to come down – probably due to its elimination and the re-synthesis of the succinate dehydrogenase enzyme.

The results of combining 3-NP with QP and SP were in accordance with the above. Only the immediate – and not the acute – effect of 3-NP on two types of open field activity, ambulatory and vertical, was significantly strengthened by QP and SP. QP acts selectively on presynaptic D_2 autoreceptors while SP has an effect on the postsynaptic D_2 receptors, (Nishino et al., 1997), leading to decreased release and action of DA in the striatum, respectively.

These results indicated that 3-NP need not be applied so long as to destroy the striatal dopaminergic neurons for a behavioural effect which is relevant in modelling HD and that the effect generated immediately is also based on dopaminergic deficit.

In contrast, neither QP nor SP caused significant alterations in open field activity versus MK-801 alone group, so MK-801 probably had an effect not the on dopaminergic, rather on the glutamatergic system.

The mechanism by which 3-NP causes neuronal loss involves the hyperactivation of glutamatergic NMDA receptors (Lee et al., 2002).

MK-801, an NMDA antagonist found to be neuroprotective at least in experimental settings (Park et al., 1988). The immediate effect of MK-801 was, in contrast to what could be expected on the basis of literature data, not opposite to that of 3-NP: the total exploratory activity decreased in the immediately, and also – although less remarkably – in the acutely treated rats. In the immediately treated rats, however, the MK-801-caused ataxia must also be considered. Application of MK-801 caused stereotypic behaviours including head weaving and uncoordinated, ataxic gait (observed also by Wu et al., 2005; Clineschmidt et al., 1982; Deutsch et al., 1997). These effects were independent of striatal DA because they could be also observed in monoamine-depleted animals (Carlsson and Carlsson, 1989). It is noteworthy that the MK-801 treated rats' total inability to stay on the rotating rod in the rotarod test was preceded by the same rats' decreased vertical activity in the OF test. Both were probably related to their ataxia, reflecting the action of MK-801 at the neuromuscular junction (Koyuncuoglu et al., 1998). Glutamate is a co-transmitter in the neuromuscular junction, acting on NMDA receptors, and blocking its effect can inhibit the muscle contraction even if the ACh mechanism is intact. In contrast, 24 h after MK-801 administration, the rats' performance did not differ significantly from control, probably due to the disappearing of the effect of MK-801 on the glutamatergic system. Based on the rats' observation during the treatments, this hypothesis is also supposable. Accordingly, the ataxia – observed in the MK-801 immediately treated rats – was over within about 8 h, probably in line with glutamatergic effect of MK-801.

In contrast, 3-NP caused minor effect on rotarod performance in both immediately and acutely treated rats. Probably a single dose of 3-NP was not enough to generate

nigrostriatal damage, the agent "only" caused deficit in the function of ion pumps, channels, and neurotransmitter systems.

The number of noise-positive responses was only insignificantly increased in the 3-NP treated rats both 30 min and 24 h after administration. The significantly shorter latency of ASR response indicated, however, the positive effect of 3-NP on the startle response. This, as DA agonists increase ASR (Davis, 1984), may have been due to DA levels increased by 3-NP.

This is consistent with the observations in the test with pre-pulse stimulus. 3-NP abolished the weak pre-pulse inhibition seen in the control group after 30 min, and did not significantly increase PPI in the 24 h test. PPI is known to be regulated by the NAC, which receives dopaminergic innervation from the ventral tegmental area (VTA). The NAC is anatomically subdivided into a core and shell region (Zaborszky et al., 1985) whereby the core belongs to that part of the striatum which is damaged on systemic application of 3-NP (Marcus et al., 2001), and in which increased DA activity leads to reduced PPI (Koch and Schnitzler, 1997; Powell et al., 2003). In addition, in the present immediate study, PPI abolished by 3-NP was reversed by SP, a D_2 antagonist, so that normal PPI was again observed in SP+3-NP treated group. This reversed PPI and increased ASR indicated also the stimulant effect of 3-NP via postsynaptic D_2 receptors. Besides, the D_2 agonist QP and the antagonist SP exerted opposite effects on 3-NP induced alteration of the number and other parameters (e.g., peak amplitude) of the noise-positive responses. When 3-NP was preceded by the postsynaptic D_2 antagonist SP, there was no significant alteration in ASR parameters compared to 3-NP group, in contrast, when QP was given before 3-NP, significant change was seen compared to both control and 3-NP alone groups. Thus it may be hypothesized that 3-NP exerts its effect via postsynaptic D_2 receptors.

In contrast, a single dose of 3-NP induced significantly shorter latency and increased peak amplitude of startle, which indicated, that the dopaminergic effect of acutely administered 3-NP was not completely disappeared by its elimination. However, its effect on DA was not observable in the PPI test, because normal PPI was seen in 3-NP acutely treated rats. Schulz et al. (1996a) described, that the plasma concentration of 3-NP come down to 10 % within 40 min. Others (Alexi et al., 1998) mentioned that 50 % recovery of enzyme activity in rat brain took 2-4 days in areas not developing lesions. In

the present study, 3-NP seemed to not eliminate completely from the plasma and/or the enzyme recovery was not perfected 24 h after its administration.

In contrast, the immediate effects of MK-801 on the ASR seemed to depend on its effect on glutamatergic system. In MK-801-treated rats, the number of noise-positive responses was significantly reduced. The primary startle pathway consists of the auditory nerve, the cochlear nucleus, and the caudal pontine reticular nucleus (PnC) (Koch and Schnitzler, 1997). The PnC is the indispensable sensorimotor interface of the pathway that mediates ASR. PnC receives direct acoustic stimuli from cochlear nucleus, a part of central auditory pathway, and the acoustic input is transmitted onto motor neurons. Neuropharmacological studies revealed that glutamate is probably the most important transmitter by which PnC neurons receive (Koch and Schnitzler, 1997) and transmit (Miserendino et al., 1993; Krase et al., 1993) the stimuli relevant to ASR. In the present study, the reduction of the ASR responses may have been caused by the direct effect of MK-801 on NMDA receptors on PnC neurons and on those innervated by them. NMDA-induced lesions in the ventrolateral part of the PnC caused, indeed, complete block of ASR (Lee et al., 1996).

DA modulators did not alter the number of noise-positive responses or other parameters of ASR induced by MK-801, suggesting that dopaminergic transmission was not, directly or indirectly, involved in the effect of MK-801.

In the present immediate study, only a slight, non-significant alteration in sensorimotor gating was observed after MK-801 treatment, and this was not further altered by QP or SP. The effect of QP itself, strong reduction of the number of noise positive responses, was similar when it was combined with 3-NP and with MK-801. So, the effect of MK-801 on PPI seemed to be completely D_2 receptor independent. There are a few reports that systemic application of MK-801 reduced the pre-pulse inhibition in rats (Kretschmer and Koch, 1998; Mansbach, 1991), but after local infusion of into the NAC enhanced it (Reijmers et al., 1995) or showed no effect on PPI (Bakshi and Geyer, 1998). The likely explanation of this is that, in the core region, the reduction of PPI is mediated by DA dependent way via projection from VTA, but in the shell region glutamate plays a key role in the regulation of PPI, which is independent from VTA (Koch and Schnitzler, 1997).

The effect of MK-801 on ASR disappeared completely in the acutely treated rats, indicating also a considerable decrease in blood concentration of the drug by that time. This is in line with Vezzani et al. (1989) who described that MK-801 reached the maximal concentrations in plasma and brain within 10 to 30 min of injection with an elimination half-life of 1,9 and 2,05 h. MK-801 is considered to bind within the NMDA-receptor complex when the receptor is activated and the ion channel is open. Following binding, the drug dissociates slowly from the receptor, thus NMDA-receptor remain inactivated only up to several hours. Accordingly, 24 h after its binding within the ion channel, its behavioural effects were not already seen.

Finally, it seems that 3-NP exerts its immediate effects via the dopaminergic system. Both QP and SP altered 3-NP-caused changes in ambulatory and vertical activities, and SP also reversed the effect of 3-NP on PPI. MK-801, an NMDA glutamatergic antagonist was, on the contrary, not able to reverse consistently the 3-NP induced changes in the spontaneous locomotor activity, or in the ASR test. Although the indirect activation of NMDA receptors is involved in the action of 3-NP on the neurons (Lee et al. 2002), the observed behavioural effects of 3-NP seem to rely, at least partly, on other mechanisms.

According to OF results, 3-NP may decrease, but to PPI test, may increase DA concentration in the striatum. The possible explanation is that different DA pathways, in different parts of the striatum, regulate locomotor activity and PPI (Sills, 1999). The dorsal striatum, comprising the caudate and putamen, is involved in the regulation of locomotion, while the core region of NAC (the main constituent of ventral striatum; David et al., 2005) mediates PPI. Reduction of DA release in the caudate-putamen (i.e, the cause of hypomotility) is mediated by an indirect inhibitory mechanism. Corticostriatal axons, mainly from the primary motor cortex, exert a dual presynaptic influence on DA release, because presynaptic D_2 receptors, identified mainly on corticostriatal axon terminals (Hersch et al., 1995), finally decrease the activation of the striatal neurons.

The aim of the work, providing the base of this Thesis, was to understand more of mechanism of the neurotoxic action of 3-NP, combined with glutamate and DA modulators, by means of neurobehavioural investigations. To know more details of the neurotoxic mechanism of 3-NP may contribute to the extension of the present, mainly

histological HD model to a more complex one including behavioural aspects. The behavioural results of the present study indicated effects in the striatum, which confirms the present histological HD model. These behavioural tests – even without histological or neurochemical measurements – show rather clearly that 3-NP exerts its effect on the dopaminergic system, possibly providing a new, behavioural aspect to the model. Behavioural methods may give opportunity to monitor the effects of agents, inducing or preventing HD-relevant damage, in a non-invasive way, contrary to the present, histological models, where the tests are not repeatable. Beyond modelling HD, the present results may gain importance in improving the therapy of other chronic neurodegenerative diseases. It is noteworthy to this point that MK-801 in our experiments failed to exert protection, in contrast to what could be expected on the basis of literature data; which indicates that, beside NMDA antagonists, DA modulators may also play a role in the therapy of neurodegenerative disorders.

Therefore, the present results may provide useful information and lead to new strategies in the modelling and treatment of chronic neurodegenerative disorders.

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7. Appendix

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